STRUCTURE-ACTIVITY STUDIES OF ION CHANNEL MIMICS

by

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We accept this thesis as conforming
to the required standard

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ABSTRACT

A sub-unit approach to synthetic ion channels is employed which allows for easy construction of a set of candidate structures. The construction set includes cores, walls and head groups. The cores are crown ethers derived from tartaric acid: 2R,3R- or 2R,3S-(18C6[1])-2,3-dicarboxylic acid, 2R,3R,11R,-12R- or 2R,3S,11R,12S-(18C6[1])-2,3,11,12-tetracarboxylic acid and 2R,3R,8R,-9R,14R,15R-(18C6[1])-2,3,8,9,14,15-hexacarboxylic acid. The crown ether is attached via an ester and a three carbon spacer to a wall unit by a thioether linkage. The walls are macrocyclic diene tetraesters derived from maleic anhydride and diols: (compounds **2, 7, 11, 21** and **27**). A Michael reaction with 3-thiopropanol produces the thioether linkage. The monoalcohol produced is converted to an iodide; the esters to the crown ethers are then obtained by nucleophilic displacement of this iodide by a crown ether carboxlate. The resulting di-, tetra- and hexaene intermediates are converted to the final compounds by addition of head groups by a second Michael reaction, with β-D-1-thioglucose, thioacetic acid or 3-thiopropanol. Using the construction set, twenty-one compounds were prepared for transport evaluation.

The transport of alkali metal cations across lipid bilayers of large unilamellar vesicles was monitored by the collapse of a proton gradient. Of the twenty one compounds surveyed, twelve of the most active were studied in

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[1]18C6=1,4,7,10,13,16-hexaoxocyclooctadecane

detail (compounds **44, 45, 46, 47, 48, 49, 50, 51, 55, 56, 62** and **63**); the other nine compounds (compounds **52, 53, 54, 57, 58, 59, 60, 61** and **64**) were not sufficiently active for full characterisation. Transport mechanisms for the twelve active compounds were investigated in parallel with gramicidin D (a channel) and valinomycin (a carrier). The transport properties examined were concentration dependence of transporter, cation selectivity and concentration dependence, and inhibition of the transport of K^+ or Na^+ by Li^+. Cation selectivities and inhibition studies proved the best tool for differentiating the channel or carrier behaviours. Carriers exhibited Eisenman cation selectivity sequences III or IV for metal ions and showed no inhibition of Na^+ or K^+ transport by added Li^+. Conversely, channels exhibited non-Eisenman cation selectivities and transport of Na^+ or K^+ is inhibited by Li^+. From comparative studies, five of the twelve compounds have strong similarities to valinomycin and are presumed to act as ion carriers (compounds **44, 47, 48, 50** and **55**). The remaining seven are similar to gramicidin (compounds **45, 46, 49, 51, 56, 62** and **63**). Within the group of ion channels, two classes of behaviour were encountered. Most compounds produce a first order decay of the imposed proton gradient (compounds **49, 51, 56** and **63**) but some showed a zero order decay in the proton gradient (compounds **45, 46** and **62**). These results are rationalised by a qualitative model which focuses on the relative rates of transfer of ion channel between vesicles, the gating or activation of the ion channel and the equilibration of a vesicle by the ion channel in an open form.

Examiners:

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To,

Mom and Dad

Unknowe, unkist, and lost that is unsought.

Chaucer

INTRODUCTION

It takes a membrane to make sense out of disorder.... To stay alive, you have to be able to hold out against equilibrium, maintain imbalance, bank against entropy, and you can only transact this business with membranes in our kind of world.

Lewis Thomas, Lives of a Cell

Natural cell membranes separate the cell contents from external solutions. At the same time, nutrients must enter the cell and waste products must be excreted. The membrane barrier must be breached if such activity is to occur. In particular, the passage of ions must be facilitated in some way since the Born charging energy for the transfer of Na^+ from aqueous solution to the centre of a 3nm thick lipid membrane[1] is ~650 kJ mol^{-1}. Transport could happen by membrane breakdown, but since the membrane protects the contents of the cell this would be catastrophic resulting in probable cell death. Transporters seek to aid ion transport in a less destructive manner. Two main mechanisms have been observed that make this movement facile. The channel mechanism exhibited by a large number of natural systems involves large protein molecules. These large transporters have been extensively studied but to date there is no detailed understanding of how individual sites within the molecule are organised or how the transport functions are promoted. Bacteriorhodopsin[2] and the acetylcholine[3] gated potassium channel receptor are the most fully studied, but even here much of the knowledge is expressed as

cartoon structures rather than in molecular detail. (**Figure 1**)

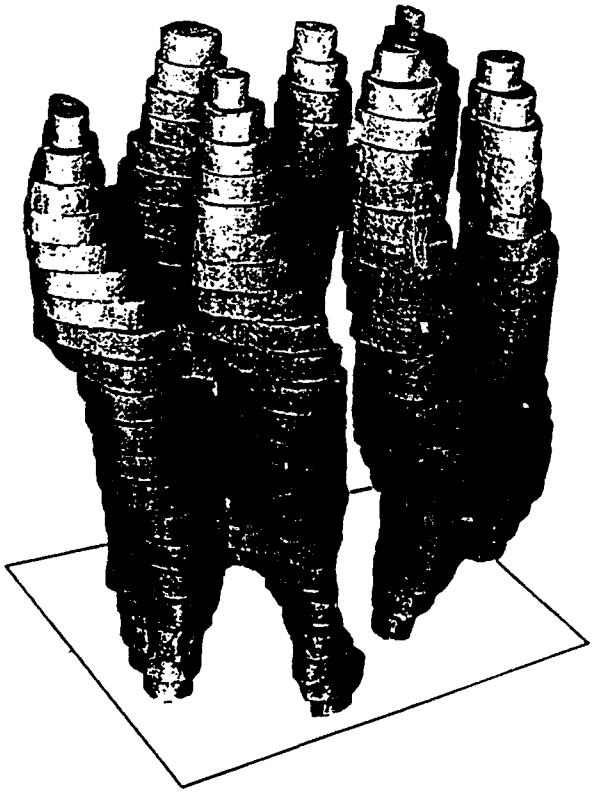


Figure 1. Bacteriorhodopsin a natural channel forming protein

The other main route to enhance membrane permeability is via the carrier mechanism. This is exhibited by many antibiotics such as valinomycin[4,5]. Carriers play no known role in normal metabolism, but a role in chemical warfare between microorganisms has been proposed[6]. Since they have a requirement for mobility, carrier molecules tend to be small. A small molecule can rapidly diffuse through a lipid membrane, selectively extract a

cation at one aqueous interface, shield the ion from the hydrophobic interior of the membrane and deposit it on the other side. Carrier ionophores such as crown ethers or cryptands are readily attainable by relatively simple synthetic strategies[7]. The mechanism of transport by these molecules has been soundly if not completely investigated[8]. The main goal of this work is not carrier-mediated transport; so I will forego further discussion of this mode of transport.

The key structural distinction between carriers and channels is that the latter are fixed within the membrane. They do not diffuse with the ion, thus they need to be comparable in size to the membrane span. This poses a significant synthetic problem since a molecule 40Å long with defined internal and external structure is required. The minimum molecular weight required for a channel mimic is estimated at 3000-5000 (g/mol). Synthetic design here is of prime importance if the goal of an active mimic is to be attained.

The simple molecule gramicidin A is probably the best natural system on which to base the design of synthetic mimics. Gramicidin A is a linear pentadecapeptide, which adopts a β helix consisting of two antiparallel monomers joined head to head by six intramolecular hydrogen bonds. This tertiary structure has been shown by crystal structures of both free[9] and metal complexed gramicidin[10]. (**Figure 2**) Unequivocal evidence of a channel mechanism is shown by single channel unit conductance measurements in black lipid membranes(BLM).[11]

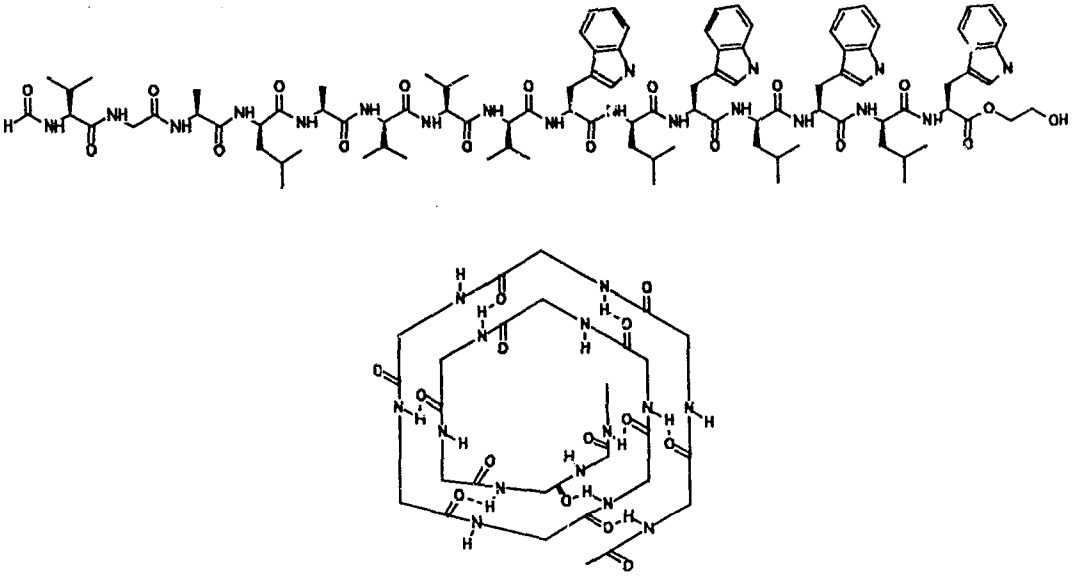


Figure 2. Top: Linear gramicidin A. Bottom: Spiral form of gramicidin A

Ion transport is proposed to occur down the axis of the β helix. The ion interacts with the amide dipoles of the peptide backbone during its transport across the membrane. From the structural units of gramicidin it should be possible to generate a set of criteria for the construction of appropriate mimics. The main requirements seen in the walls of gramicidin channels are that a mimic should have a large number of dipolar groups that will facilitate ionic passage through the hydrophobic membrane. The lumen or interior has a diameter of 4 Å, meaning that the structure is selective for small monovalent cations. For any mimic to have well defined ion selectivities it must also have a defined fixed diameter, or restriction point to act as a selective filter.

The obvious first choice in mimic design would be structurally modified polypeptides that might mimic gramicidin directly. One of the best examples is that of Stankovic, Heinemann and Schreiber[12], which links two units of gramicidin A by means of a dioxolane linker. This removes the dynamic constraints imposed by tail to tail dimerisation in gramicidin A. These mimics give some simple insight to the gating mechanism of ion conduction. In an active channel a column of water is believed to connect the internal and external phases. When the conformation of the linker dioxolane flips, a carbon of the dioxolane ring inserts into the column of water and causes a discontinuity. This creates what the authors term "flicker" states in the single channel ion conductances. (**Figure 3**)

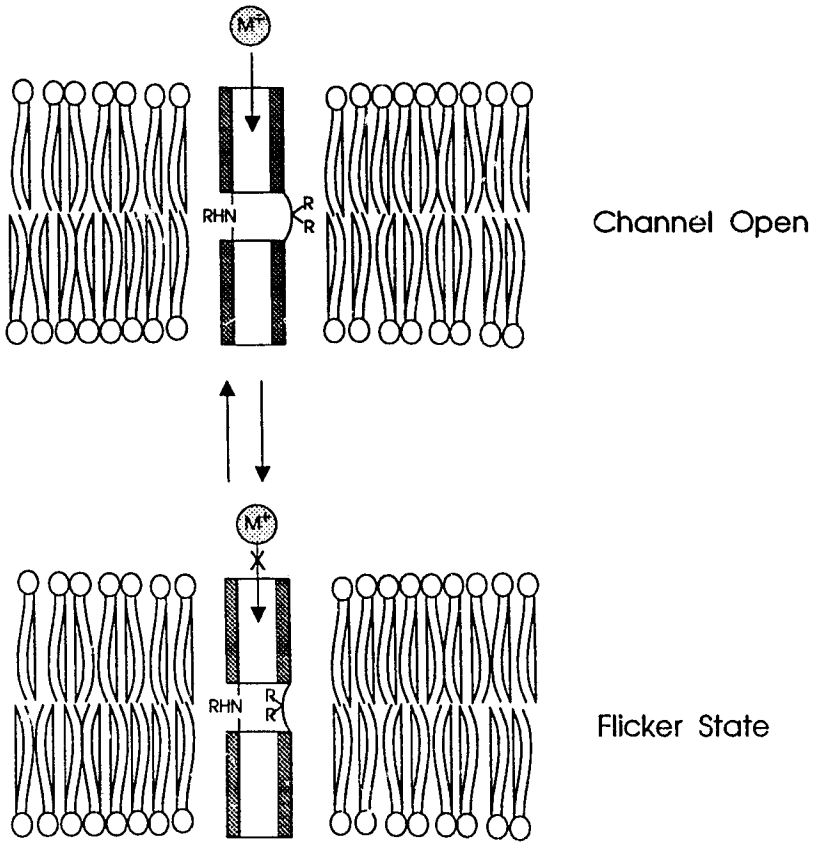


Figure 3. The Stankovic channel mimic: dioxolane linked gramicidin A

Other peptide derived gramicidin mimics have been studied[13,14] but they tend to form large aggregates rather than discrete units. These large aggregates "permeablise" the membrane but they have reduced selectivity when compared with the smaller and better defined gramicidin channel. These large aggregates have a greater resemblance to melittin[15,16] an active component of bee venom. Melittin permeablises membranes by forming aggregates which disrupt large areas of the lipid bilayer membrane.

The first rational design of a channel mimic was carried out by Tabushi[17]. His system is based on β-cyclodextrin, which has four long alkyl chains attached. (**Figure 4**) Tabushi's compound has a degree of rigidity within its structure which imposes some constraints on its association and assembly within a membrane. Tabushi proposes that the active compound arises as a result of tail to tail dimerisation of two β-cyclodextrin units. The cycodextrin supplies the binding sites required by a metal ion to bring it from the aqueous phase and into the hydrophobic environment of the lipid membrane. The alkyl chains and amide linkage then help direct the metal through the membrane to the other cyclodextrin and eventually to the internal aqueous phase. The alkyl chains have limited functionality so presumably this is an unfavourable process. Another possible mode of action involves aggregation and the formation of larger defect structures within the membrane. These defects could be water filled crevasses rather than structurally distinct units. Activity in the case of this compound was assessed by the ability to permeablise egg phosphatidyl choline vesicles to cobalt or copper. The transport efficiency was monitored using a UV active metal complexing dye. (Tiron)

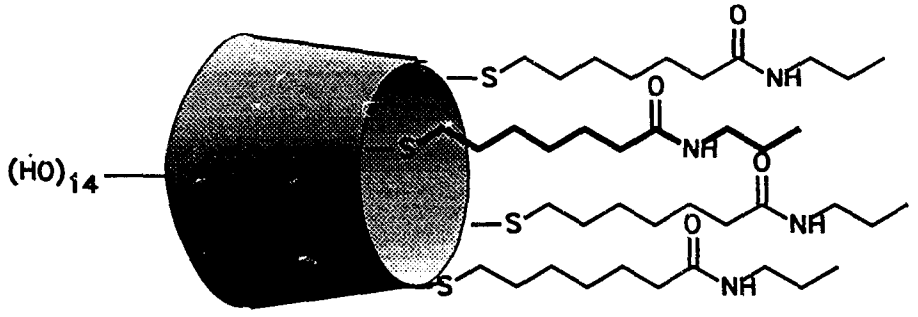


Figure 4. The Tabushi cyclodextrin based channel

The approach of Nolte[18-21] is simple but elegant. The predisposition of isocyanide polymers to form α helices of one turn every four units was used to produce four linked channels. Crown ether rings were linked to isocyanide monomers, which on polymerisation formed chains of approximately forty repeating units that would contain ten complete turns. This forms four stacks of sandwiched crowns with an overall length of 4nm. (**Figure 5**) This compound fulfils the general criteria above, it has a great number of ion complexing sites and is rigid. The efficacy of the compound was measured by its ability to permeablise dihexadecyl phosphate vesicles to cobalt ions. Transport was monitored by internal UV absorptions of the dye 4-(2-pyridylazo)resorcinol monosodium salt. The definitive evidence that is quoted in defense of a channel mechanism over that of carrier is the activation energy. The observed activation energy of the Nolte compound is 24 kJ mol^{-1}; gramicidin A has an activation energy of 20.5-22.5 kJ mol^{-1} in the same membrane system. Carrier mediated transport however has a much higher

activation energy; a value of ~ 100 kJ mol^{-1} was cited.

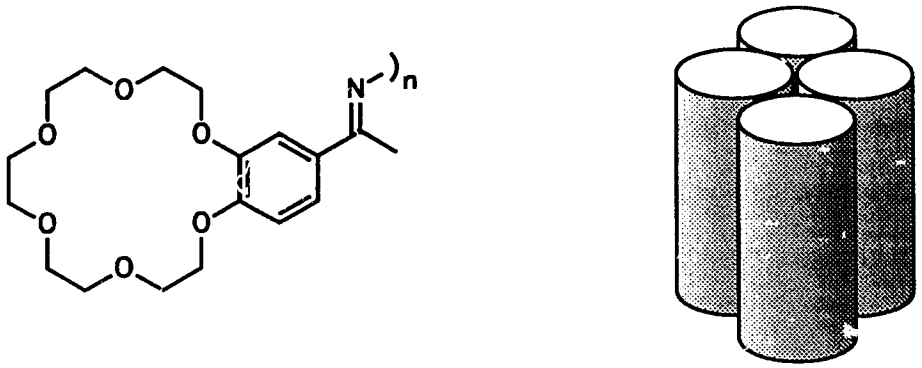


Figure 5. The Nolte crown ether isocyanide polymer channel

Gokel[22] has developed an exceptionally simple synthetic strategy which results in a very active mimic. The compound consists of three macrocycles, linked by alkyl chains. (**Figure 6**) The mimic is believed to allow ion movement by a site to site ion hopping mechanism similar to gramicidin. He proposes that two macrocycles are situated at either membrane aqueous interface and the remaining macrocycle at the membrane mid-plane. This mimic then has three sites to facilitate an ion "hop" across the membrane. The number of sites is much lower than in gramicidin and the compound is not rigid, making this on first glance seem a poor model. The efficacy of the compound to permeablise vesicle membranes proves this assumption to be incorrect, and shows perhaps the minimum requirements of a mimic. Dynamic $^{13}Na^{+}$ NMR spectroscopy was used as the tool for monitoring ion flow across

large unilameller vesicle membranes prepared from egg lecithin. As proof for the channel mechanism employed by this mimic, Gokel compares its transport properties with a structurally similar carrier. The carrier is merely the central unit with alkyl arms but lacking the other two macrocycles.

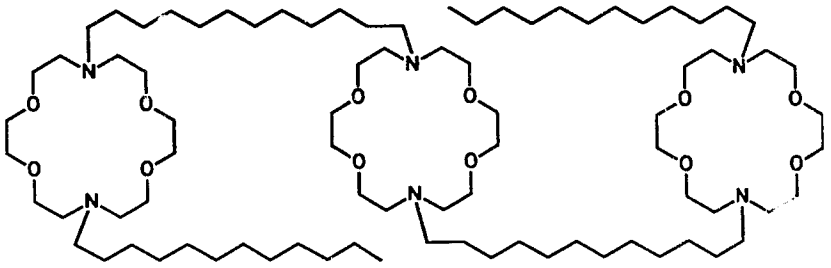


Figure 6. The Gokel *tris* macrocyle based channel

Much suggestive work, but as yet no functional mimics, has come from Lehn's group. The solid phase crystal structure of monomeric tartaramide crown ethers display a channel like structure in the solid state[23]. The crown cavities lie perpendicular to an axis that passes through their centre of symmetry. Two other main approaches have also been proffered by Lehn. One involves the linkage of multiple crowns by short spacers to form a rigid tube. This particular avenue has apparently faltered synthetically; a three unit system is the largest prepared to date[24]. The other approach, similar to ours involves a central annulus linked to bundles of ionophilic chains[25]. (**Figure 7**) The synthesis has been described but no transport results have yet appeared.

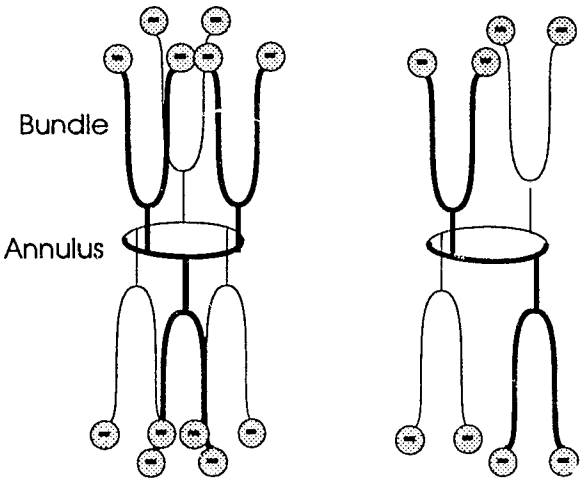


Figure 7. The "chundle" approach to channel design by Lehn

A few other active compounds have found their way to the literature. These compounds apparently do not act by a carrier mechanism. Rather they form active units by some type of association. These compounds more directly resemble Amphotericin B [26,27](a pore) and its mode of action than they do gramicidin (channel). The term *pore* is used here to represent a range of mechanistic possibilities involving aggregates of several molecules which create defects and other nonspecific structures. *Channel* is used as a label for compounds that closely resemble gramicidin in their mode of action: a defined, ion selective structure. From now on whenever the terms *pore* or *channel* are used they will refer to these arbitary distinctions.

Kunitake[28] takes a somewhat different tack to the design of compounds that permeablise vesicle membranes. His compounds closely resemble the synthetic membrane in which they are incorporated. These molecules function because of the property of phase separation on a microscopic scale. The oil in water approach is very simple and takes advantage of a known thermodynamic property exhibited when two dissimilar solvents are mixed. The boundary regions between separate phases are essentially more hydrophillic than the bulk phase and may result in localised membrane breakdown. The induction of an aqueous canal could allow the transport of solute molecules across the membrane. The ability to form such pores was evaluated by the ability to transport hydroxide (or counterport protons) to an entrapped pH dependent fluorescent dye (riboflavin). (**Figure 8**)

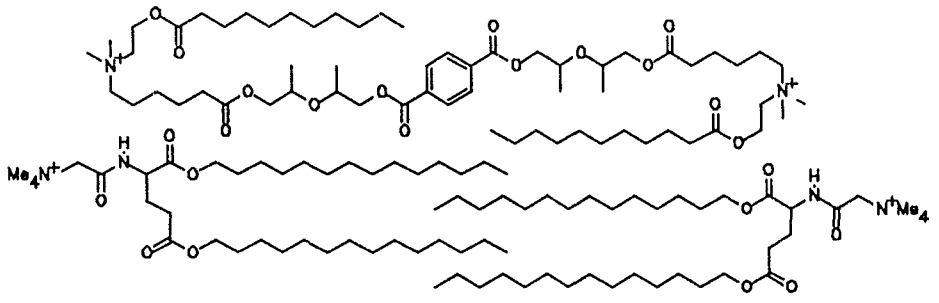


Figure 8. Top: Kunitake pore former. Bottom: Kunitake lipid molecules for scale

The work of Menger[29,30] has similar roots. His molecule works by self association resulting in either membrane defects or in the creation of a localised ordered system within the membrane. The efficacy of the compounds

is established by their ability to collapse a pH gradient imposed across a membrane measured by the quenching of an entrapped fluorescent dye. (**Figure 9**) Note that Menger's target compounds were inactive; it is only the precursors which successfully collapse trans-membrane pH gradients.

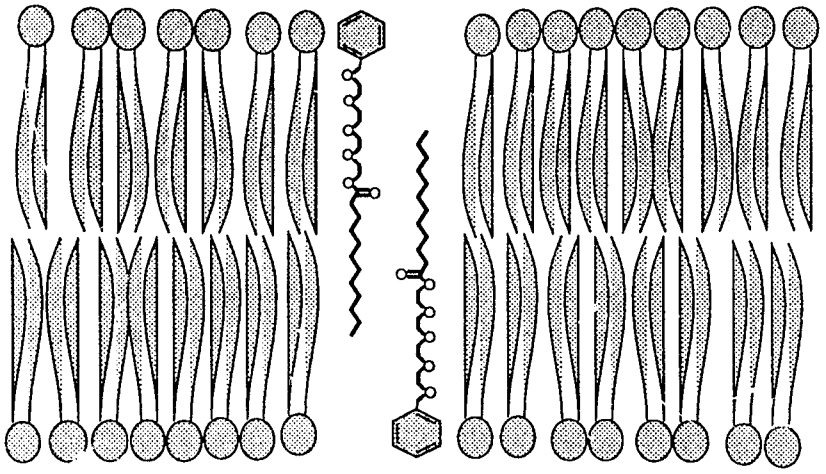


Figure 9. Cartoon of the Menger pore former inserted into a schematic bilayer

The system designed by Fuhrhop[31-34] has reduced synthetic requirements. A synthetic monolayer membrane which is only 20 Å thick halves the span required of an active compound. Furhop's membrane system consists of double headed amphiphiles dispersed in water to form monolayer vesicles. These vesicles are normally sealed to lithium efflux. The active compound monensin pyromelatate accelerates lithium efflux as assessed by a gel chromatography experiment. (**Figure 10**)

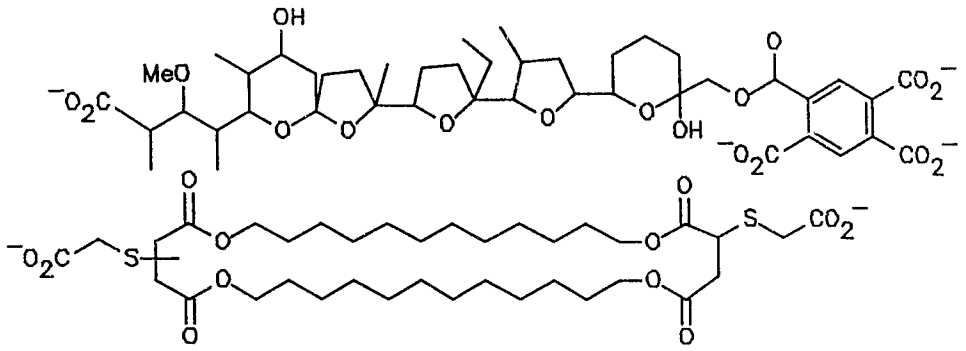


Figure 10. Top: Monensin pyromeletate. Bottom: Fuhrhop's synthetic membrane for scale.

The very fact that each of these structurally non related molecules has some modicum of success, must make us realise that we are a long way from understanding the factors that control ion transport by channels, pores or defects.

Early work carried out by Zojaji and myself [8] was directed to active compounds for use in the Fuhrhop synthetic membrane system. Our synthetic compounds were expected to be a better probe of the intermolecular forces involved than monensin pyromelatate. (**Figure 11**) This approach offered some success and the compounds did permeablise the monolayer vesicles. A combination of both synthetic and transport evaluation problems eventually conspired to terminate this approach. The key problem was vesicle leakage and our subsequent work has sought to avoid significant background leakage wherever possible.

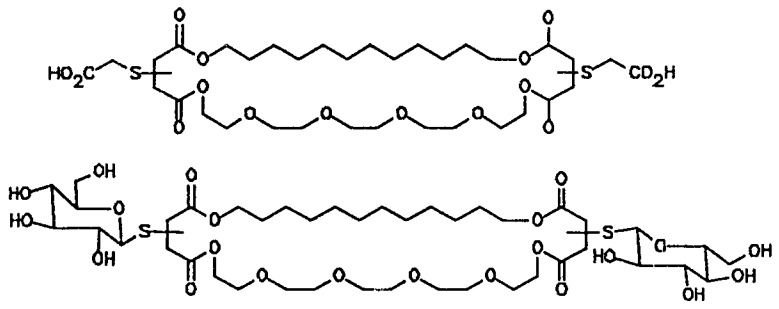


Figure 11. Two pore formers for Fuhrhop synthetic membranes

Our solution to the vesicle leakage problem was to change to vesicles prepared from egg phosphatidyl choline. Compounds that can span this lipid membrane need to be 40Å long, twice the length required in the Fuhrhop system. This length is achieved by linking two macrocyles.[35,36] Zojaji has prepared a suite of fourteen such "pore formers", which he has shown to be active in egg phosphatidyl choline vesicles.[37] **(Figure 12)**

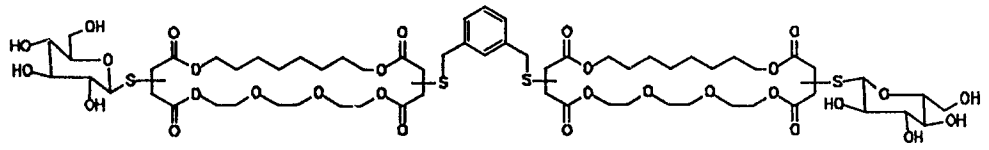


Figure 12. A pore former based on linked maleic anhydide derived macrocycles for phospholipid bilayer membranes.[37]

Work in our group to create and explore ion channel mediated transport started with Dutton, Carmichael and Swan. The design structure consists of a central crown ether bearing "wall" units to span the membrane and "head" groups anchored in the aqueous phases. **(Figure 13)** The efficacy of this

compound was measured by its ability to collapse a pH gradient imposed across egg phosphatidyl choline vesicles, monitored by the disappearance of fluorescence intensity due to entrapped fluorescein.[35,38]

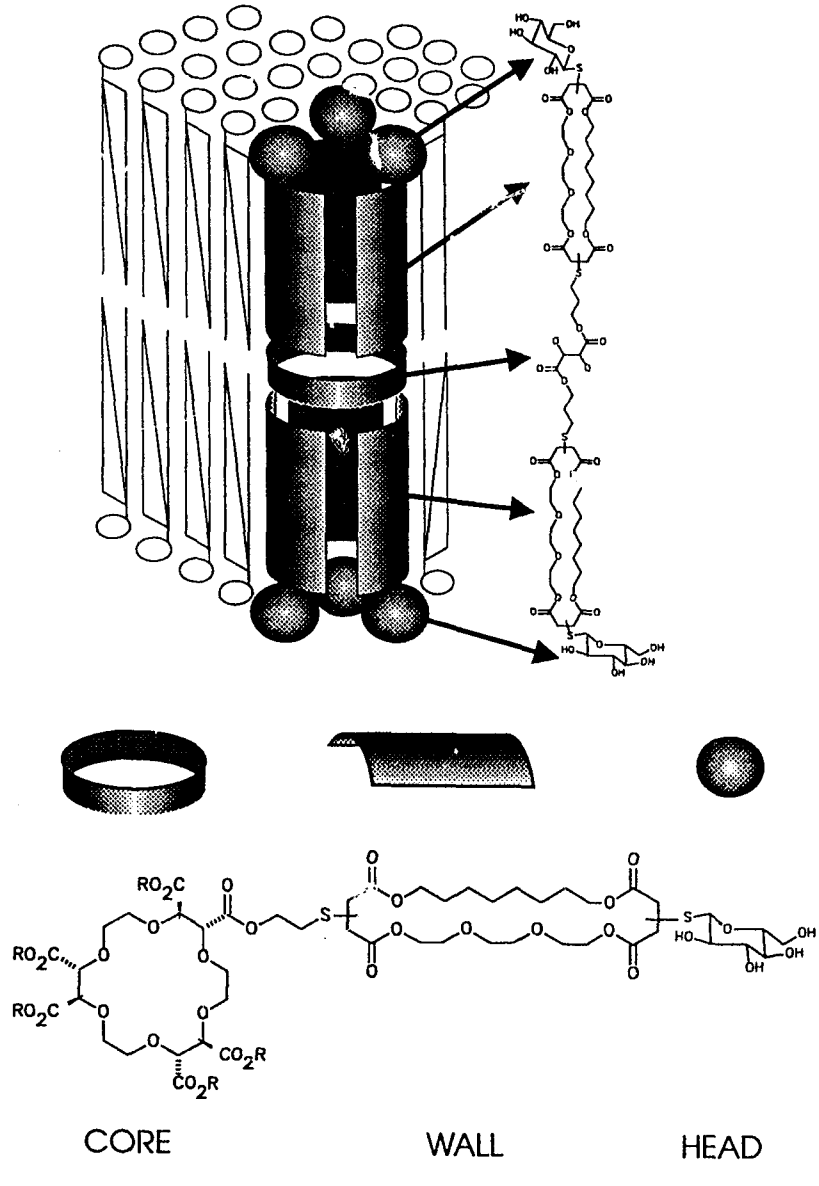


Figure 13. The channel design: structure assembled from building blocks.

Considering the design in more detail, it can be seen that it pivots around the choice of the central crown. The particular crown used was synthesised by Dutton. This tartaro crown (2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylic acid) could stabilise the metal ion at the membrane midplane. More importantly the tartaric acid units have a strong conformational preference for an *anti* disposition which imparts rigidity, and directs the attached walls perpendicular to the plane of the crown. Rigid wall units derived from macrocyclic tetraesters that had polar faces to facilitate and direct ion movement from the aqueous phase through the membrane, were appended. The length of such walls was about 20Å, since they were needed to span half the natural bilayer thickness. The choice of polar heads was made as a compromise between two factors. The polar head had to be able to insert into the membrane easily, but must be favourably partitioned into the aqueous phase.

The compound prepared required relatively simple synthetic manipulations for a target of its size. The wall units came from a modification of the Furhop macrocyclic tetraester forming reaction used in the preparation of the compounds of **Figure 10**. Linkage to the crown ether hexaacid synthesised by Dutton was achieved using a simple acid chloride plus alcohol ester condensation reaction in the presence of base. The alcohol linker unit was incorporated into the wall using a Michael reaction of the wall alkene

with a thiol, in this particular case 2-thiopropanol. The head group, in this case 1-thioglucose, was incorporated using the same Michael reactivity of the wall alkenes. The head groups were added last to ensure that no interfering functional groups were present in the wall when it is added to the crown.

From the design structure it can be seen that a "tinkertoy tool box" could exist from which a great number of mimics could be assembled. The possible units could have walls that are of different length or they could have variable polarity or have a greater number of ionophilic sites than illustrated in **Figure 13**. The crown ether which is the crux of the synthesis could include examples with fewer linker sites, producing channel mimics with fewer arms. The cavity size of the crown could also be modified, possibly resulting in some differential ion selectivities. If the core size is great enough, the compounds may display no selectivity. This would be similar to the observed properties of large aggregated pore structures. The head group balance is a factor which may affect the action of the channel mimics. If the head groups reside in the membrane rather than the aqueous phase then an inactive compound could result. If the head group becomes too large or polar then the compound might not be able to insert through the membrane.

The goal of this particular study was to explore these structural variations based on the earlier work. This expansion was done by exploring the activity of a basis set drawn from possible structure variations mentioned above. With a detailed investigation of these structural variants a deeper

understanding of the factors involved in the creation of a channel with designed function can be envisaged.

It has become clear that rational design can create channel mimics that have efficiencies as high as those present in nature. But unlike nature, the ability to modulate this transport in a known and regulated manner is still in its infancy. This is something that nature has been master over since the first membrane surrounded the first cell. To be able to rationally design, not just an active compound, but one that has some predicted function is the goal. This can only be achieved if the model chosen has the ability to grow with experimentally determined truths. For a model to possess these attributes it must be prepared from structural subunits that allow easy modification. The hope of this work and other work like it is not to answer all these questions but simply make it possible to start asking some of the right ones.

SYNTHESIS

The retrosynthetic analysis of complex natural products allows complete evaluation of all possible syntheses. With the design criteria set down for channel mimics, a retroanalysis is useful only so far as it gives ideas for possible sub-unit construction. The final goal is not fixed as it is in natural product synthesis, but can change to accommodate both setbacks and advances on the synthetic front. (**Figure 14**) From this retroanalysis it can be seen that three basic units exist: cores, walls, and head groups. This allows flexibility amongst components, but simplicity and reliability in the final assembly. The "tinkertoy set" could include, for cores: rings of varying size, rings to which more or less walls can be attached, or rings that direct the walls equatorial rather than axial. The walls could possess polar or non-polar functionality, have varying length and varying breadth. The heads could be neutral or charged and, large or small.

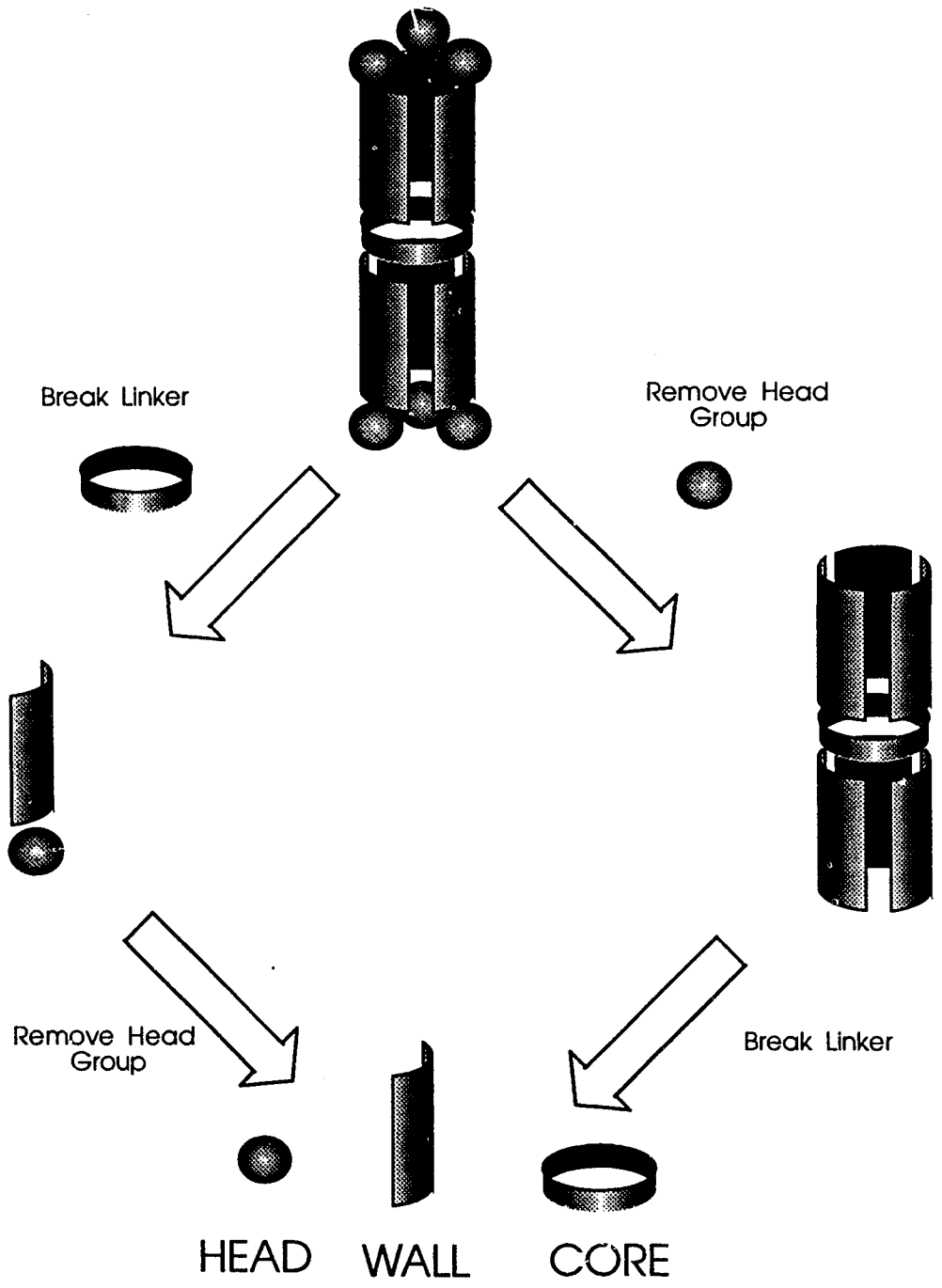


Figure 14. Schematic retroanalysis of channel mimics

The preferred basic structural subunit for walls are macrocycles derived from a synthesis proposed by Fuhrhop[30]. The Fuhrhop macrocycle is prepared from maleic anhydride and 1,12-dodecanediol in two steps and in a reasonable yield of 82%. (**Figure 15**) This macrocycle is of particular interest since the preferred conformation as calculated by MM2 results in a linear macrocycle very similar in shape to the illustration in the schemes. The hydrocarbon chains adopt all trans conformations. This unit could insert into lipid with hydrocarbon portions similar to hydrocarbon segments of phospholipid.

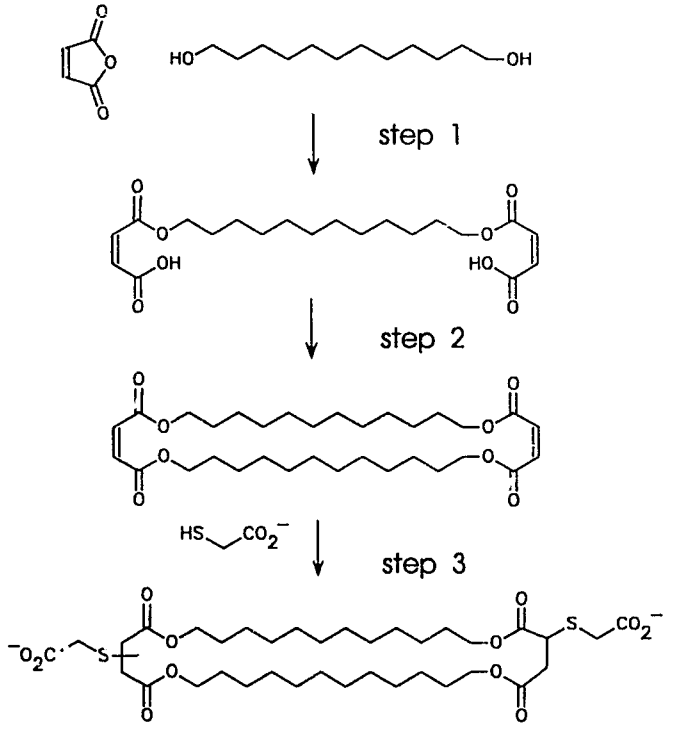


Figure 15. Fuhrhops bolaphile synthesis

The cores are tartaric acid based crown ethers, due to both the metal ion

complexing abilities and the structurally rigid conformation of the tartaric acid sub units to which walls can be appended. The carboxylic acids provide sites which are directed perpendicular to the crown ether plane. The carboxylates have an *anti* disposition shown by crystal structures of salt complexes[39] and Karplus analysis of the ^{1}HNMR of related derivatives[40]. In some cases (*meso*-tartaric acid) this conformation results in distortion of the macrocyclic crown ether[40]. For the supply of five of these tartaro derived crowns, I must thank previous workers (Dutton/ Carmichael/ Swan/ Wotton/ Hocking). (**Figure 16**)

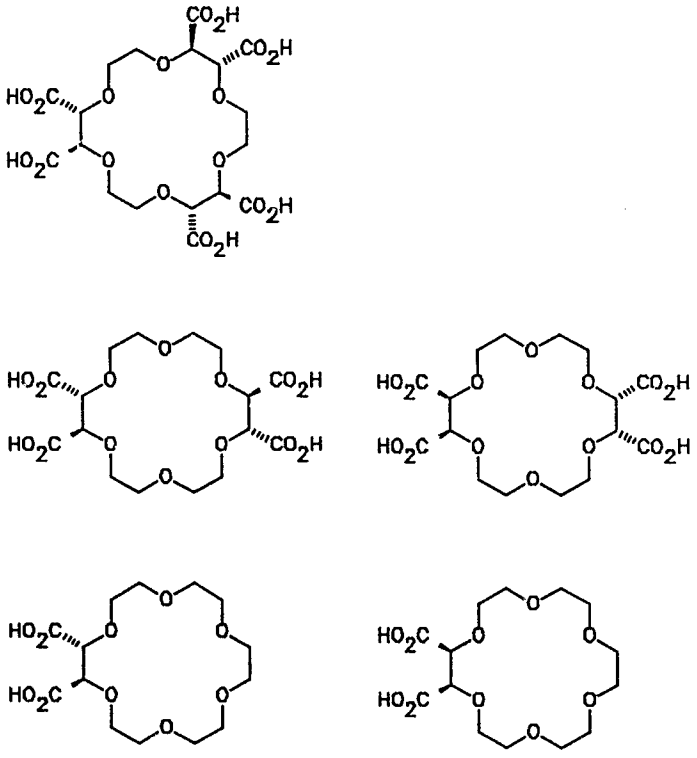


Figure 16. Five tartaric acid derived crown ethers

A link must be made between the carboxylic acid of the crown ether and the wall macrocycle. At the crown ether, linkage could be by amide or ester. The latter was chosen to avoid uncontrolled amide hydrogen bonds within the lipid. Sulphur linkage to the tetraester macrocycle is chosen on the basis of the work of Fuhrhop[31]. The thioethers are prepared by the very facile Michael reaction of thiols to the conjugated alkene of the macrocycle.

Head groups could be added at two stages of assembly. In either case, Michael addition to the tetraester macrocycle would be facile. Head group addition preceding linkage to the crown was not attempted due to the complicating factor of multiple functional groups.

On initial scrutiny the Fuhrhop synthesis looked like a simple synthetic route from which many different macrocycles could be prepared for use as walls. This surmise was proven correct for the symmetric macrocycles, all of which are simply prepared in modest yields. (**Figure 17**) Compound **24** has been described by Fuhrhop[31].

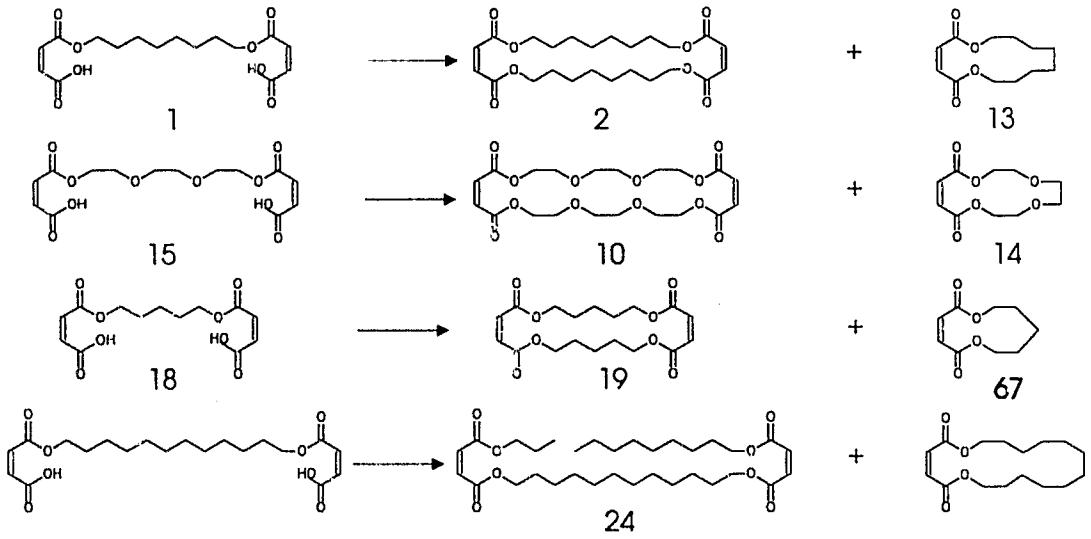


Figure 17. Symmetric macrocycles derived from maleic anhydride.

However, once the synthesis is pushed further and a second structurally different diol is added in step two other than that used in step one then things begin to go awry. Taking another look at **Figure 15**, one realises that a very important factor was neglected namely the byproducts. Initially it was thought that these byproducts were simply the formation of oligomers. Extensive work by Carmichael and Swan on this system and by Zojaji on a macrocycle based on 1,12-dodecanediol and pentaethylene glycol, has shown these other products to be smaller macrocycles consisting of one maleic anhydride and one diol unit in twice the yield of the desired macrocycle. These "half" macrocycles are the result of "tail biting" transesterification. In the symmetrical case this is not a major problem since the mixture is relatively simple.

Compound **2** was prepared in two steps derived from the method of

Furhop. Compound **1** was obtained quantitatively and used without further purification in the macrocyclisation step. A mixture of compound **2** and **13** was produced. Trituration followed by successive recrystallisation removed **13** from the mixture to give **2** as colourless needle-like crystals in 12% yield. Analysis of the reaction mixture and product by ^{1}HNMR did not help characterisation as both **2** and **13** have identical overlapping spectra. Mass spectral analysis was the most useful in establishing the purity of the compound **2** (M+1, 453), since even trace amounts of compound **13** (M+1, 227) showed strongly in the mass spectra obtained. Elemental analyses were obtained, but do not give any idea of the purity since the compounds are oligomers.

Compound **19** was also prepared in two analogous steps. The required diacid (compound **18**) was obtained quantitatively and used without further purification. The second macrocyclisation step produced compounds **19** and the "half"-macrocycle; successive recrystallisation removed the byproduct from the mixture to give **19** as colourless rhombohedral crystals in 10% yield. Analysis of the reaction mixture and product by ^{1}HNMR did not help characterisation as both **19** and the smaller oligomer have identical overlapping spectra. The ^{13}CNMR spectra were more informative. Both compounds have similar spectra but the alkyl ester carbon (CO_2CH_2) for compound **19** is at 62.4 ppm and that for compound **18** is at 62.3 ppm. Mass spectral analysis was the most useful in deciding the purity of compound **19** (M+1, 369), since even trace amounts of the "half"-macrocycle (compound **67**)(M+1, 185) showed up in the mass

spectra obtained.

Compound **10** was prepared in one pot without isolation of the diacid (compound **15**). Purification of the mixture of **10** and **14** required column chromatography followed by trituration of individual fractions to give **10** as a white solid in 7.6% yield. As with compound **19**, ^{1}HNMR spectra were of little use. ^{13}CNMR spectra were more useful: the alkyl ester carbon (CO_2CH_2) for compound **10** is at 64.5 ppm and that for compound **14** is at 63.9 ppm. As with compound **19**, mass spectral analysis was the most useful in deciding the purity of compound **10** (M+1, 461), since even trace amounts of compound **14** (M+1, 231) showed up in the mass spectra obtained.

The "non-symmetric" macrocycle (compound **7**) proved to be a much more taxing problem. (**Figure 18**) Careful chromatography worked to a limited degree to give a mixture of **14** and the desired compound **7** in a 2:1 ratio. Gel (size exclusion) chromatography was attempted on this mixture but proffered little success as **14** and **7** seem to have comparable hydrodynamic radii. Utilising Kuglrhor distillation the mixture could be separated. Compound **14** is fractionated at 120°C under a pressure of 10^{-3}mmHg. Compound **7** does distill, albeit at a high temperature (220°C at 10^{-3}mmHg) and on standing after distillation crystallises as fine needles. As was finally practised, chromatography was merely used to remove **2**, **13** and the methane sulphonic acid catalyst from the crude reaction product. The mixture of **7**, **10** and **14** was distilled to give **7** in an isolated yield of 6.4%.

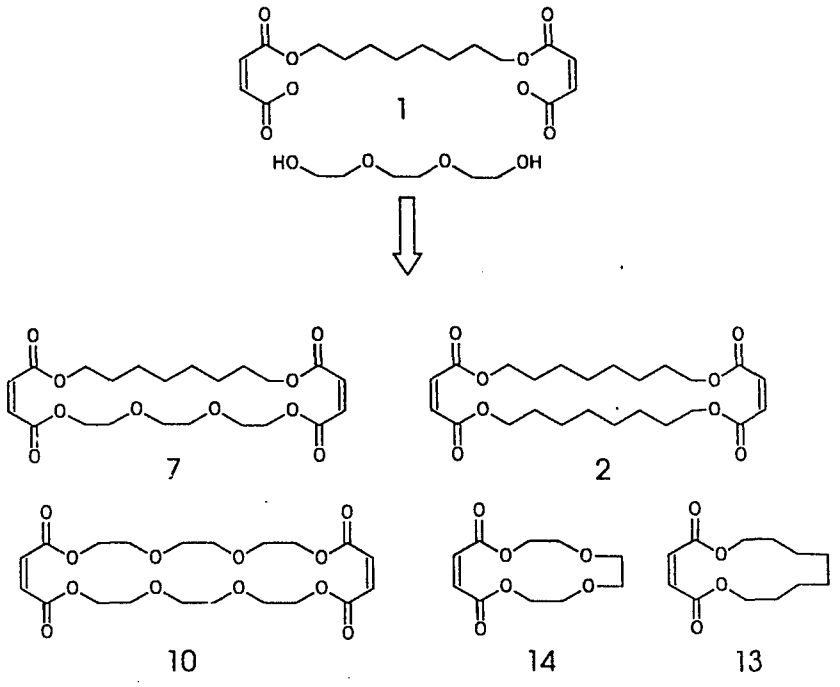


Figure 18. Synthesis of the disymmetric macrocycle compound **7**. Macrocycles formed: the desired compound **7** with **2** and **10** plus the "half"-macrocycles **14** and **13** as side products.

As with compounds **2**, **10** and **19** ^{1}HNMR spectra are of little use in the analysis of the crude mixtures. However, at an advanced purification step when a mixture of **14** and **7** is procured, integration becomes a useful tool in characterisation. For pure **7** the alkyl ester (CO_2CH_2) and alkyl (CH_2) integration ratios should be 8 and 12 respectively. A mixture contaminated with **14** has a ratio in which the proportion of ester increases with respect to the alkyl fragment. The ^{13}CNMR spectrum, as with the other macrocycles, differentiates the alkyl ester carbon (CO_2CH_2) of compound **7** at 64.1 ppm and

compound **14** at 63.7 ppm. Mass spectral analysis, as before, was the most useful technique in deciding the purity of compound **7** (M+1, 457), since even trace amounts of compound **14** (M+1, 231), **13** (M+1, 227), **10** (M+1, 461) or **2** (M+1, 453) showed up in the mass spectra obtained.

The next step in the construction of "channel mimics", is perhaps the simplest. The conjugated alkenes of the macrocycle undergo very facile Michael reactions with thiol nucleophiles. (**Figure 19**) In the first instance, Michael addition was used to add a "linker" to the macrocycle. This unit will ultimately join the wall to the core. Examples of the manipulations about this "linker" are illustrated in **Figure 19**. When the reaction is carried out under mild conditions, (50°C for 1 hour) the expected mixture of products is obtained with up to 50% isomerisation of the remaining Z alkene to the E isomer. Under more stringent conditions, (reflux for 1 hour 15 minutes) the remaining alkene of compound **3** is completely isomerised to the E form. Both ^{1}HNMR and ^{13}CNMR spectra are utilised in the characterisation of the isomerisation; the E isomer has an alkene signal at 6.8 ppm in the ^{1}HNMR and 133.5 ppm in the ^{13}CNMR spectra, the Z isomer signal is at 6.2 ppm in the ^{1}HNMR and 129.6 ppm in the ^{13}CNMR spectra. The scheme shows the Z,Z isomer of compound **2** and **7** transforming to the Z isomer of compound **3**. This pictoral representation will be used throughout the Thesis to conserve space on the structures and to simplify presentation. For experimental convenience the E

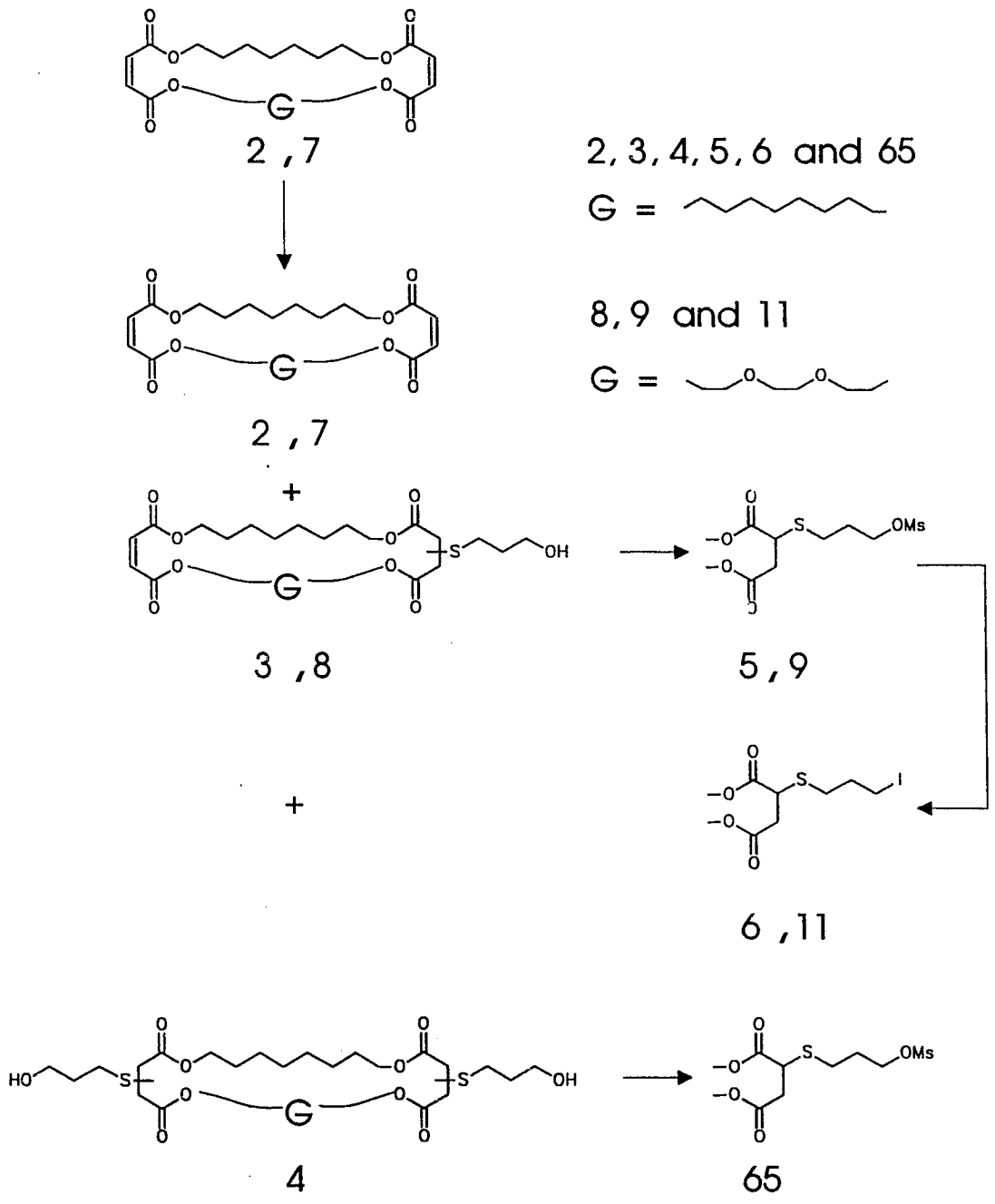


Figure 19. The addition of 3-thiopropanol to compounds **2** and **7**, and the subsequent manipulations

forms were prepared wherever possible to simplify product mixtures; in any event, the alkene stereochemistry is lost in the final targets.

For the thioether linkages ^{1}HNMR was useful at the purification step. In the mono adduct isolated from the statistical mixture of unreacted and doubly reacted macrocycle, the integration ratio for the ester (CO_2CH_2) and Z alkene (CH=CH) should be 8 to 2. Unreacted macrocycle results in an increase in the amount of alkene; likewise the presence of the doubly reacted product results in a decrease in the proportion of alkene. The most consistent spectral measure obtained throughout this work is the ^{13}CNMR spectra of the thioether linkage. For all the "linkers" and macrocycles used the $SCHCH_2$ (41.8 ppm) and $SCHCH_2$ (36.6 ppm) carbons of the macrocycle vary less than 1 ppm.

The mechanism of the Z to E isomerisation seems to involve the reversible addition of the base, in this case piperidine, to the alkene. (**Figure 20**) Reversibility of the base addition reaction is not one hundred percent and some piperidine adduct is always obtained. This is not a major problem for most of the macrocycles, since it is formed in trace amounts and is easily removed by column chromatography. With compound **10**, the base addition was a major contaminant and was not successfuly removed by column chromatography. The solution to this problem was to use the bulky base 2,2,4,4-tetramethylpiperidine rather than normal piperidine. The hindered base did not add to the macrocycle as was shown by the lack of isomerisation of the Z alkene. The product from **10** has a signal at 6.2 ppm in the ^{1}HNMR

spectrum and 129.7 ppm in the ^{13}CNMR spectrum. (*cf.* the Z isomer; 6.8, and 133.5 ppm for the ^{1}HNMR spectrum and ^{13}CNMR spectrum, respectively.)

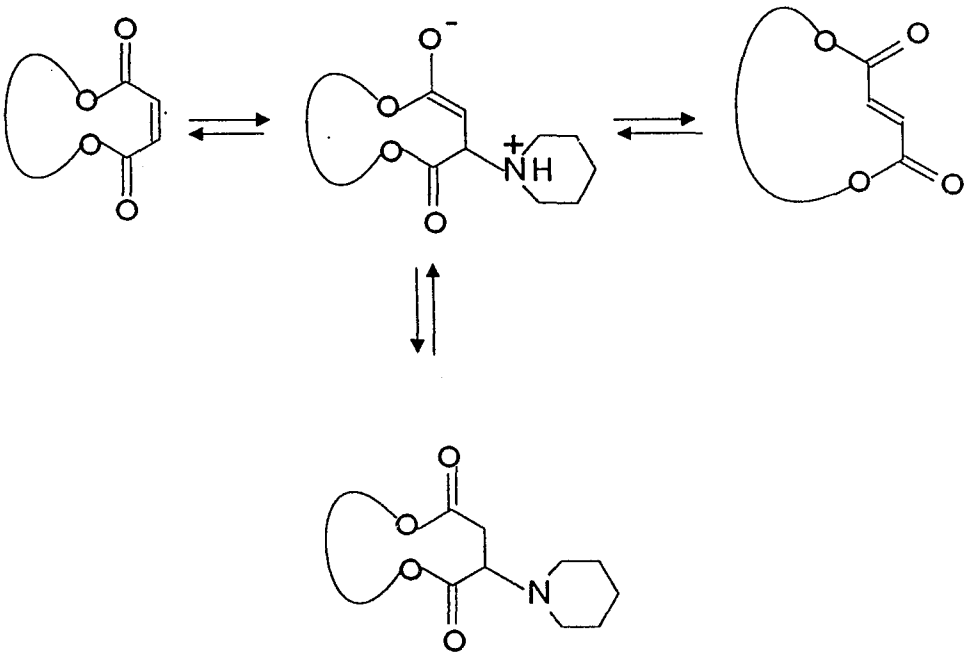


Figure 20. Isomerisation of double bond from Z to E caused by piperidine.

Previously the "walls" had been linked to the crown, using the acid chloride of the crown (Dutton and Carmicheal) and the alcohol of the 2-thioethanol linker. (**Figure 21**) Given the knowledge of the compounds at the time, the method seemed to work well. However, when I repeated the reaction the products I obtained troubled me. The crown ether methine signals appeared in the ^{13}CNMR spectrum as either more than one peak, a broad mass or were not observed at all.

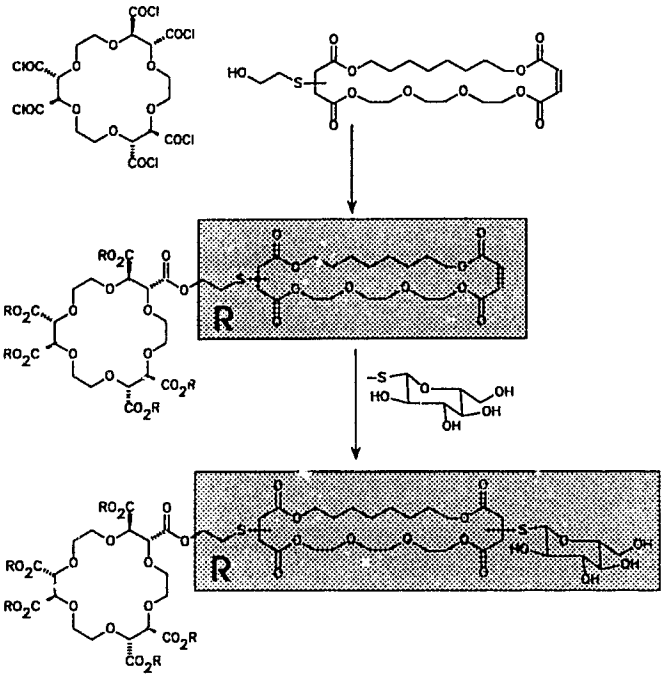


Figure 21. Formation of the ester linkage by condensation of alcohol and acid chloride. Both Z and E isomers of the alkene were present.

Part of the reason the other workers (Dutton and Carmichael) were not troubled by these broad signals I believe goes back to the thiol addition mentioned earlier. Their "linkers" were a mixture of Z and E isomers so it was expected that the methine should be broad as the molecular symmetry of the product is reduced. Since I observed this feature when the linker was the single E isomer, the chiral contiguity of the crown must have been destroyed. It is easy to see how epimerisation could occur at the methine centre. Since base is present (Et_3N) hydrochloric acid could be eliminated from the crown. The ketene formed would then react with the alcohol. But the reaction is no longer stereo specific: the alcohol could attack the ketene from the pro R or pro

S face, resulting in epimerisation of the crown methine. (**Figure 22**). The reaction was also attempted in the absence of base. This approach still resulted in a product with more than one methine signal. Without the base the reaction equilibrium becomes progressively more unfavourable as the amount of mineral acid builds up. Extra peaks for the methine are then ascribed to only partial reaction, not all of the crown's acids having been esterified. As with epimerisation this lowers the symmetry of the product.

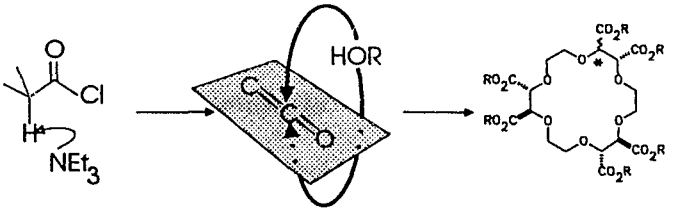


Figure 22. Isomerisation of crown ether methines caused by piperidine.

The nucleophilic carboxylate method espoused by Kellogg[41] was then investigated.

$$RCO_2H + RBr \xrightarrow[DMF]{CsCO_3} RCO_2R$$

This particular method could not work directly with the 2-thioethanol since replacing the alcohol with a leaving group makes these compounds sulphur mustards. The solution was to use the homologue of 2-thioethanol, 3-thiopropanol. This could be prepared from 3-bromopropanol or obtained commercially.

Model systems were exploited in order to explore the chemistry. Reaction of 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid in DMF at 70°C over crushed molecular sieves with both butylbromide and octylbromide results in the esterification of the crown carboxylates. The ^{13}CNMR spectra of the purified products show crown methines (*C*HO) at 78.9 and 78.9 ppm and alkyl ester carbons (CO_2CH_2) at 66.5 and 63.0 ppm respectively. Similar products were obtained when similar procedures were used with the corresponding mesylates. When the mesylated compound **65** is reacted with butyric acid under analogous conditions to those employed with the simple alkyl mesylates, then the dibutyrate ester compound **66** is formed. (**Figure 23**) Analysis of the ^{13}CNMR spectrum shows the butyric ester carbon (CO_2CH_2) at 173.6 ppm and the alkyl ester carbon (CO_2CH_2) at 62.6 ppm.

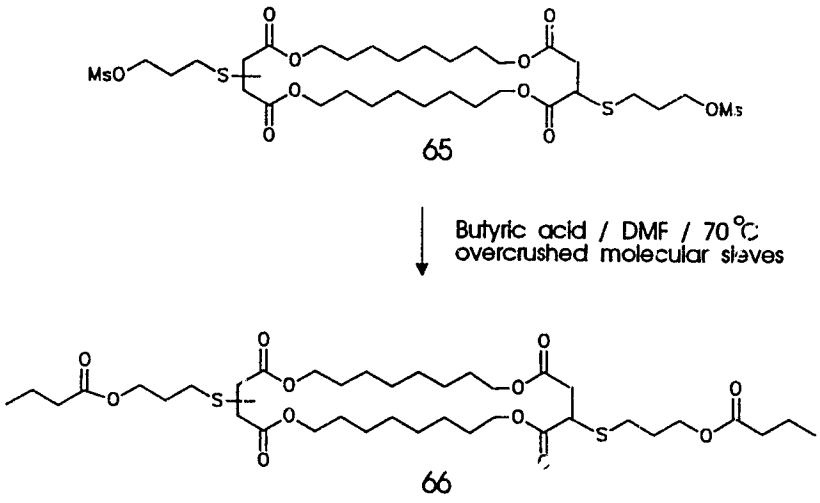


Figure 23. Reaction of compound **65** with butyric acid to form the dibutyrate ester (compound **66**)

When compound **9** was used with 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid instead of a simple alkyl derivative the reaction failed. Unreacted **9** was recovered as the major component of the reaction mixture. The main problem and the one directly responsible for all other problems associated with the reaction, was the insolubility of the crown carboxylate salts formed from cæsium carbonate. The insolubility of the cæsium salt should have been expected since the crowns crystallise very readily from aqueous solution on the addition of alkali metal ions.[36] (**Figure 24**)

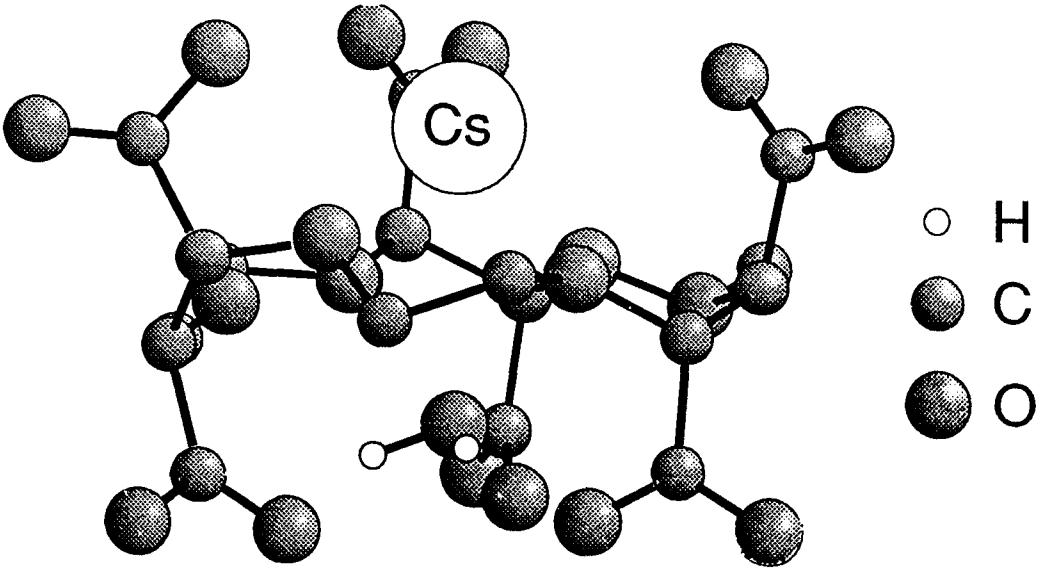


Figure 24. The crystal structure of the cæsium salt of 2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylic acid.

Initial attempts to broach the problem revolved around making the leaving group of the linker better (bromide and then iodide) in the hope that a very active system here would make up for the insolubility of the cæsium carboxylates. (**Figure 19**) Reaction of the alcohol (compound **3**) with phosphorus tribromide in order to make the bromide directly failed. Substitution of the bromine atom via nucleophilic displacement of the mesylate (compound **5**) was attempted. Characterisation of the product was a problem as the mass spectrum was unobtainable, the triplet due to the C***H***$_2$Br of the linker was obscured in the ^{1}HNMR spectrum, and the ***C***H$_2$Br was not present or hidden in the ^{13}CNMR spectrum. The more reactive iodide compound **11** was then made by nucleophilic substitution of the mesylate by iodide in 75% yield. Compound **11** was easily characterised by the CH$_2$C***H***$_2$I triplet at 3.2 ppm in the ^{1}HNMR spectrum and the ***C***H$_2$I carbon at 4.4 ppm in the ^{13}CNMR spectrum.

Reaction of the iodide (compound **6**) with 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid in DMF with excess cæsium carbonate led to the disapearance of **6**; only **2** was isolated from the reaction mixture. (**Figure 19**) In the anâlogous series derived from macrocyle **7** with one equivalent of base, the product isolated was the formate ester, (**Figure 25**) characterised by a singlet (C***H***O) in the ^{1}HNMR spectrum at 8.1 ppm and a signal at 165.3 ppm in the ^{13}CNMR spectrum (***C***HO DEPT). The formate was a result of the iodide reacting with dimethyl ammonium formate

impurity in the DMF.

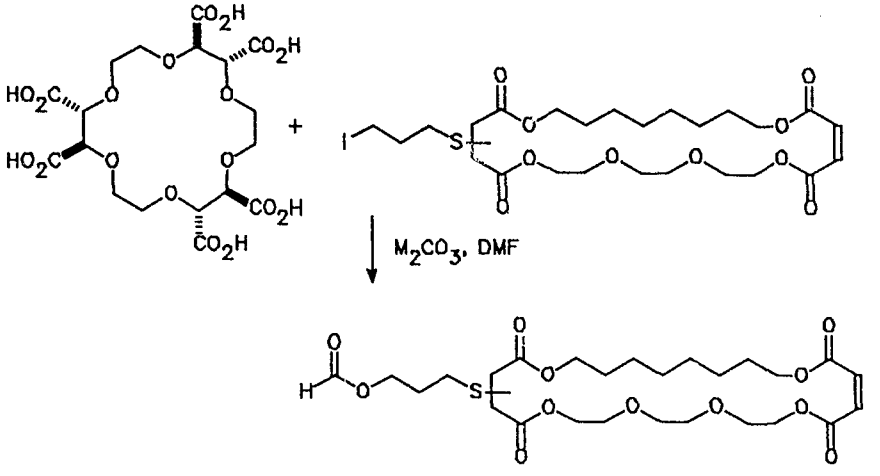


Figure 25. Formation of a formate ester from compound **11** by reaction with dimethylammonium formate in the DMF solvent.

A survey of the cæsium 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacycloctadecane-2,3,11,12-tetracarboxylate solubility showed it was extremely insoluble in THF, DMF, DMA, DMSO and HMPT. Other alkali metal salt complexes, sodium and potassium were also insoluble. The reaction mixture of **6** and 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid with tetramethylammonium hydroxide as the base in DMF at 60°C was homogeneous but due to the dimethylammonium formate impurity most of the isolable product was formate ester. In N,N-dimethylacetamide (DMA) the product was the acetate ester. When the reaction of **6** and 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid with tetramethylammonium hydroxide as the base was carried out in DMSO at 70°C for 12 hours the desired product **30** was obtained. A similar reaction of **11**

gave **36**. (**Figure 26**) Reaction times of greater than 12 hours result in progressive hydrolysis of the product ester. The products of hydrolysis were witnessed in the ^{13}CNMR spectrum of **30** or **36** as the appearance of a signal due to a hydroxymethyl carbon ($\mathbf{C}H_2OH$) at 60.9 ppm.

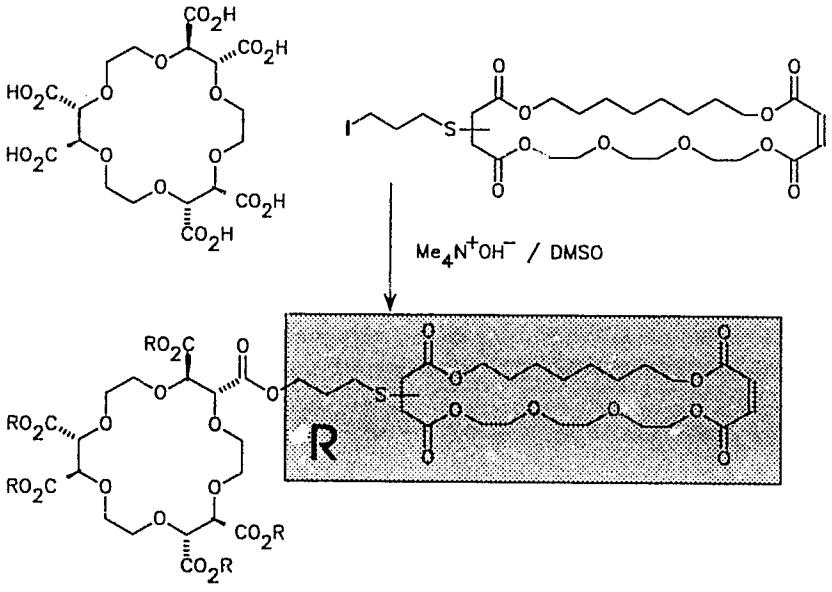


Figure 26. Formation of the desired hexaester (compound **36**) using tetramethyl ammonium hydroxide and the iodide compound **11** in DMSO at 70°C.

The desired products **30** or **36** show very sharp methine signals at about 80.0 ppm in the ^{13}CNMR spectra when compared with the broad methine signal in the spectrum of the product from the acid chloride reaction. An ester carbon ($CO_2\mathbf{C}H_2$) at 63.5 ppm is also evident. (**Figure 27**)

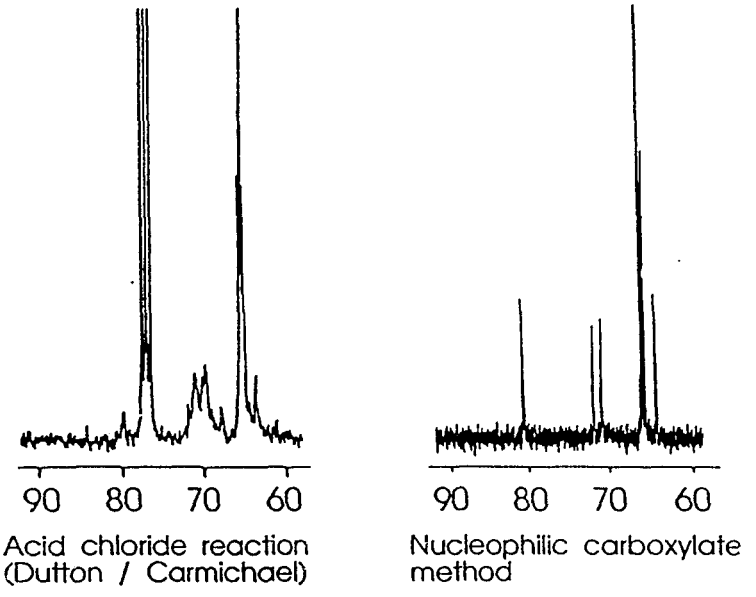


Figure 27. The appearance of the crown ether methine in the ^{13}CNMR spectra produced by the carboxylate and acid chloride ester forming reactions.

Out of a possible suite of twenty five compounds, fifteen were finally prepared. All of these compounds have a sharp ester carbon signal of the crown ($\mathbf{C}O_2CH_2$) at ~169 ppm, sharp methine carbon (**C**HO) signal at ~80.0 ppm and characteristic alkyl ester carbon signal of the linker ($CO_2\mathbf{C}H_2$) at ~63.5 ppm in the ^{13}CNMR spectra. (**Figure 28**)

In the next section the ^{13}CNMR spectra for compounds **30**, **31**, **34**, **36**, **38** and **39** are given with assignments. For comparison the ^{13}CNMR spectral assignments of precursors are also given. The spectra for these compounds show sharp carbon resonances all of which have been assigned. The simplicity of these spectra for compounds that have high molecular weights is ascribed to the high degree of molecular symmetry; symmetry imparted by the crown ether core.

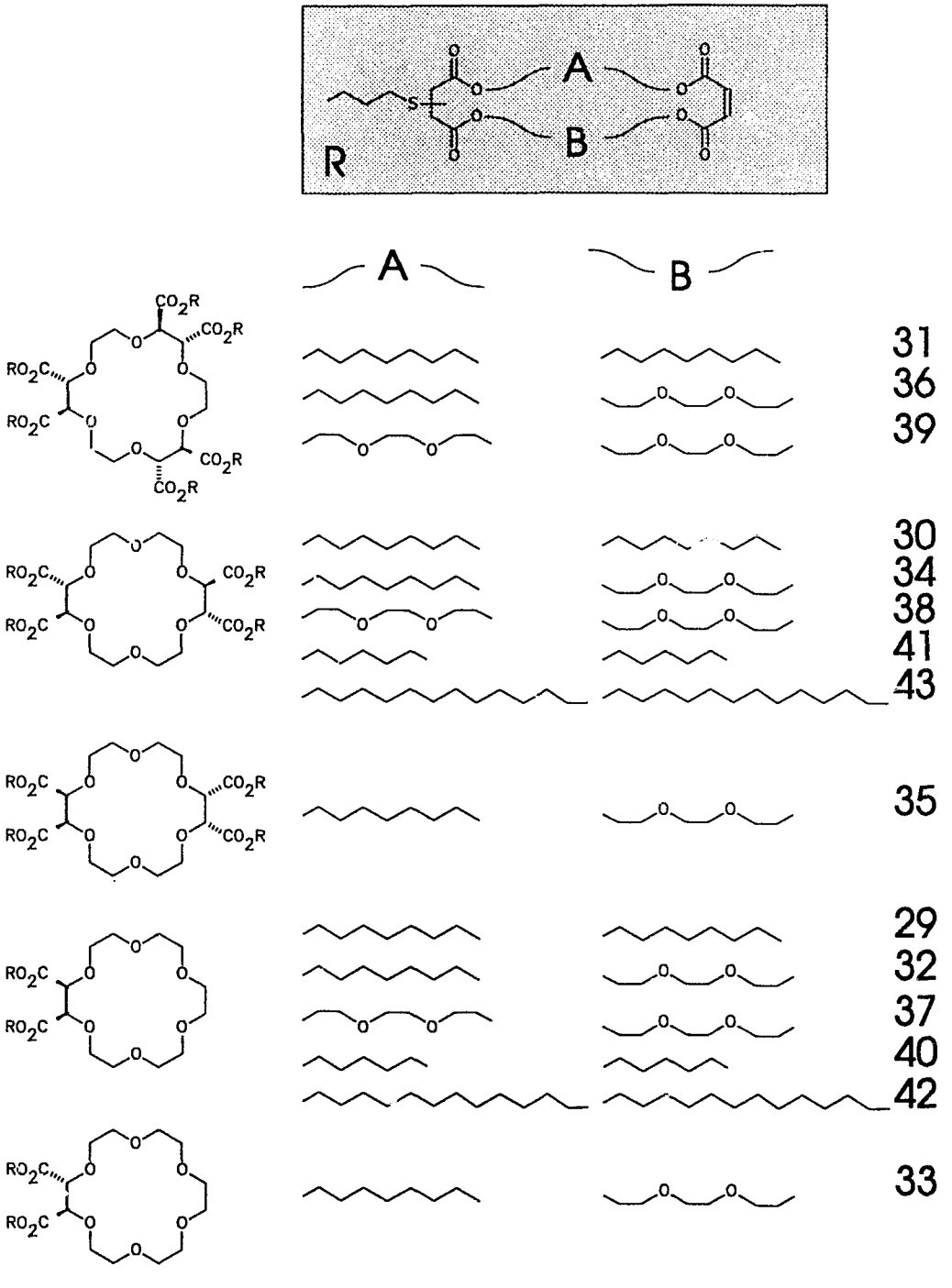


Figure 28. The fifteen molecules synthesised

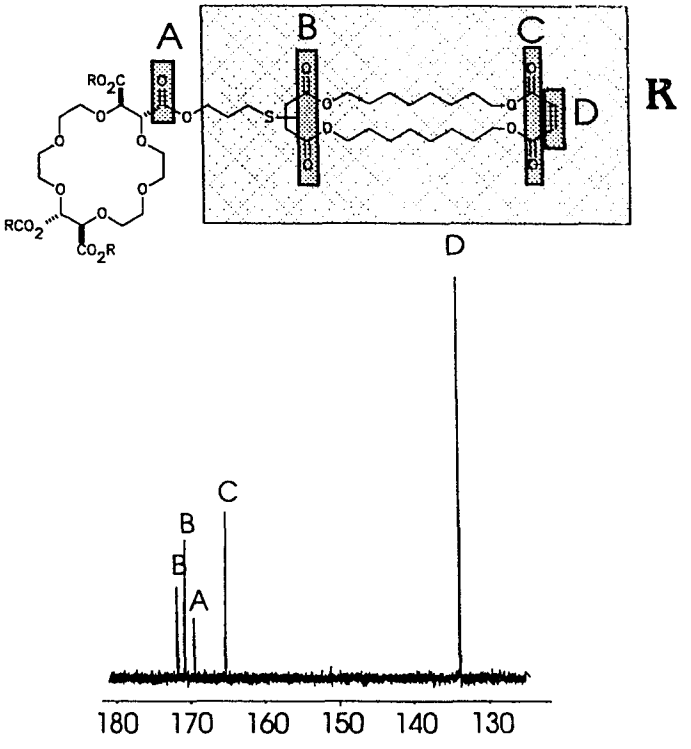


Figure 29. The ^{13}CNMR spectrum for compound **30** as the E isomer from 180-130ppm.

Table 1. Comparison of the ^{13}CNMR spectrum of **30** with those obtained for compounds **3** and **2** from 180-130ppm.

Assignment	Compound **2**	Compound **3**	Compound **30**
Carbonyl (C)	165.2	165.0	165.2
Carbonyl (B)	-	171.7 170.6	171.7 170.8
Carbonyl (A)	-	-	169.5
Alkene (D)	129.6 (Z)	133.5 (E)	133.8 (E)

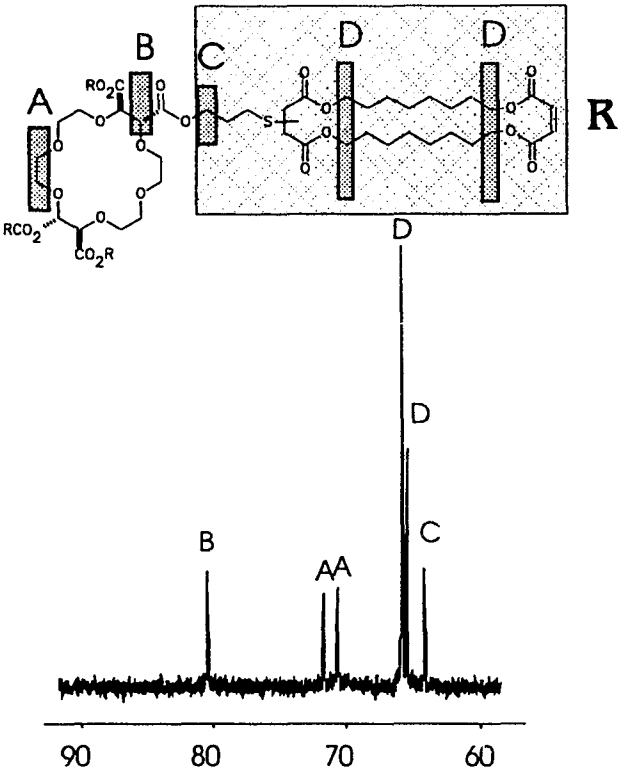


Figure 30. The ^{13}CNMR spectrum for compound **30** as the E isomer from 90-60ppm.

Table 2. Comparison of the ^{13}CNMR spectrum of **30** with those obtained for compounds **3** and **2** from 90-60ppm.

Assignment	Compound **2**	Compound **3**	Compound **30**
Crown CH_2 (A)	-	-	71.5 70.5
Crown CH_1 (B)	-	-	80.3
Ester linker CH_2 (C)	-	-	63.9
Ester macrocycle (D)	65.3	65.3 64.8	65.6 65.5 65.3 65.2

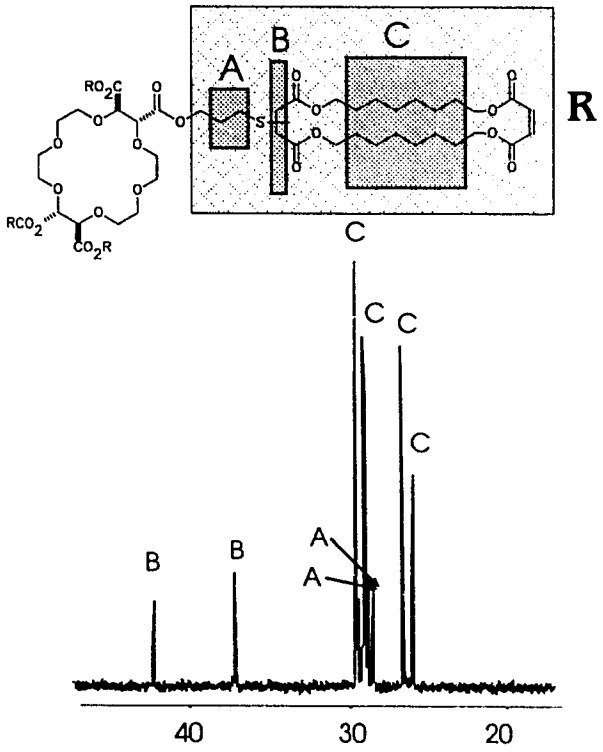


Figure 31. The ^{13}CNMR spectrum for compound **30** as the E isomer from 50-20ppm.

Table 3. Comparison of the ^{13}CNMR spectrum of **30** with those obtained for compounds **3** and **2** from 50-20ppm.

Assignment	Compound **2**	Compound **3**	Compound **30**
Linker CH_2 (A)	-	31.8 28.1	28.6 28.2
CH_2CHS (B) CH_2CHS (B)	-	41 36.6	41.9 36.9
Macrocycle CH_2 (C)	29.1 28.4 25.8	28.9 28.4 26.0 25.3	29.8 29.2 28.8 28.7 26.4 25.7 25.6

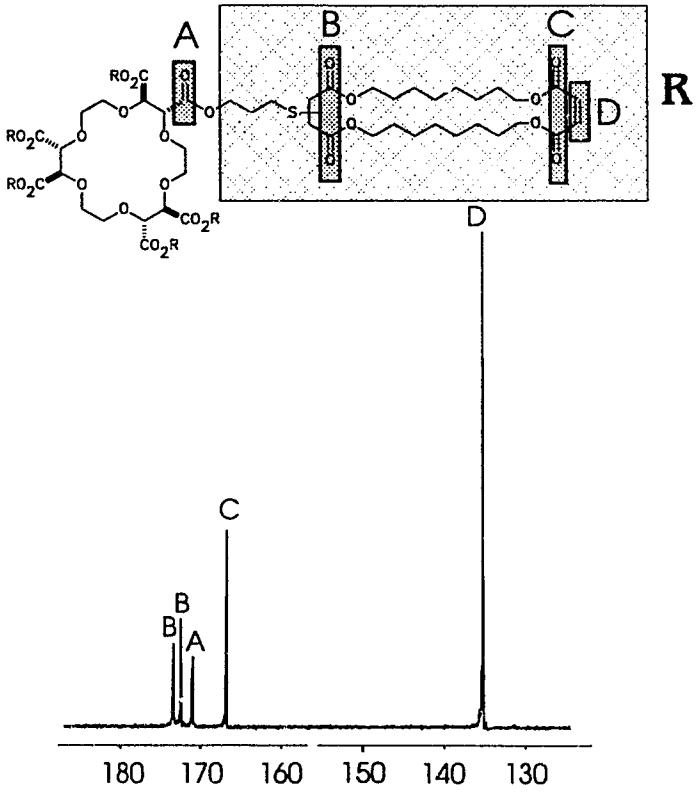


Figure 32. The ^{13}CNMR spectrum for compound **31** as the E isomer from 190-120ppm.

Table 4. Comparison of the ^{13}CNMR spectrum of **31** with those obtained for compounds **3** and **2** from 190-120ppm.

Assignment	Compound **2**	Compound **3**	Compound **31**
Carbonyl (C)	165.2	165.0	165.2
Carbonyl (B)	-	171.7 170.6	171.7 170.8
Carbonyl (A)	-	-	169.4
Alkene (D)	129.6 (Z)	133.5 (E)	133.7 (E)

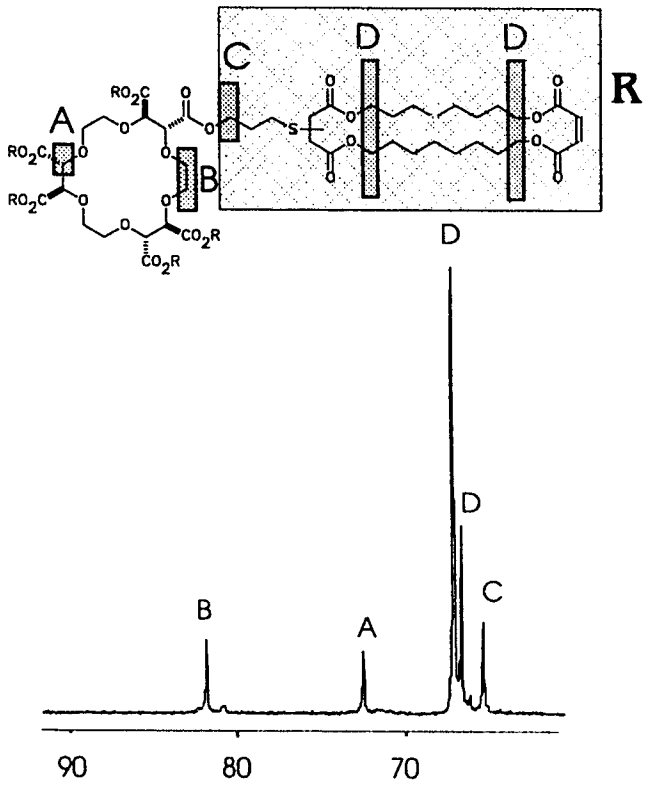


Figure 33. The ^{13}CNMR spectrum for compound **31** as the E isomer from 90-60ppm.

Table 5. Comparison of the ^{13}CNMR spectrum of **31** with those obtained for compounds **3** and **2** from 90-60ppm.

Assignment	Compound **2**	Compound **3**	Compound **31**
Crown CH_2 (A)	-	-	70.9
Crown CH_1 (B)	-	-	80.2
Ester linker CH_2 (C)	-	-	63.9
Ester macrocycle (D)	65.3	65.3 64.8	65.6 65.5 65.1

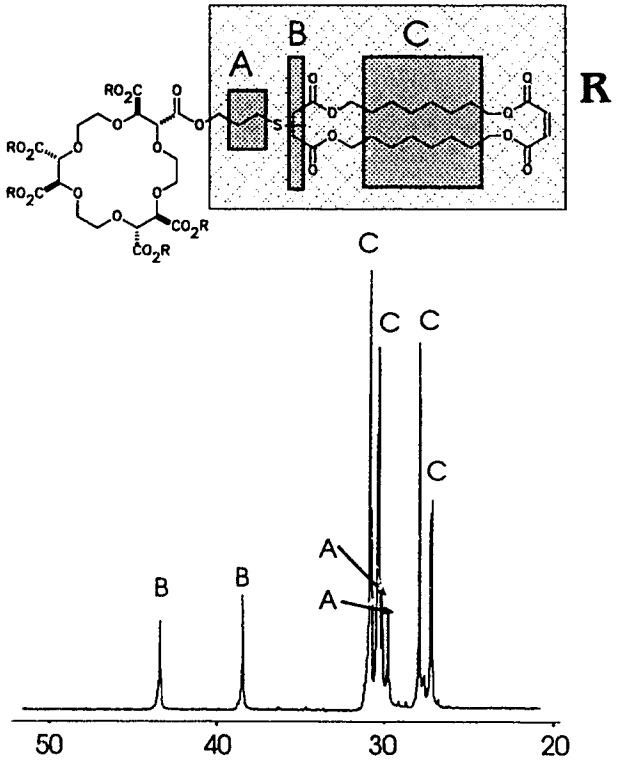


Figure 34. The ^{13}CNMR spectrum for compound **31** as the E isomer from 50-20ppm.

Table 6. Comparison of the ^{13}CNMR spectrum of **31** with those obtained for compounds **3** and **2** from 50-20ppm.

Assignment	Compound **2**	Compound **3**	Compound **31**
Linker CH_2 (A)	-	31.8 28.1	28.6 28.2
CH_2***C***HS (B) ***C***H_2CHS (B)	-	41.7 36.6	41.8 36.9
Macrocycle CH_2 (C)	29.1 28.4 25.8	28.9 28.4 26.0 25.3	29.3 29.3 29.2 28.7 26.4 25.7 25.6

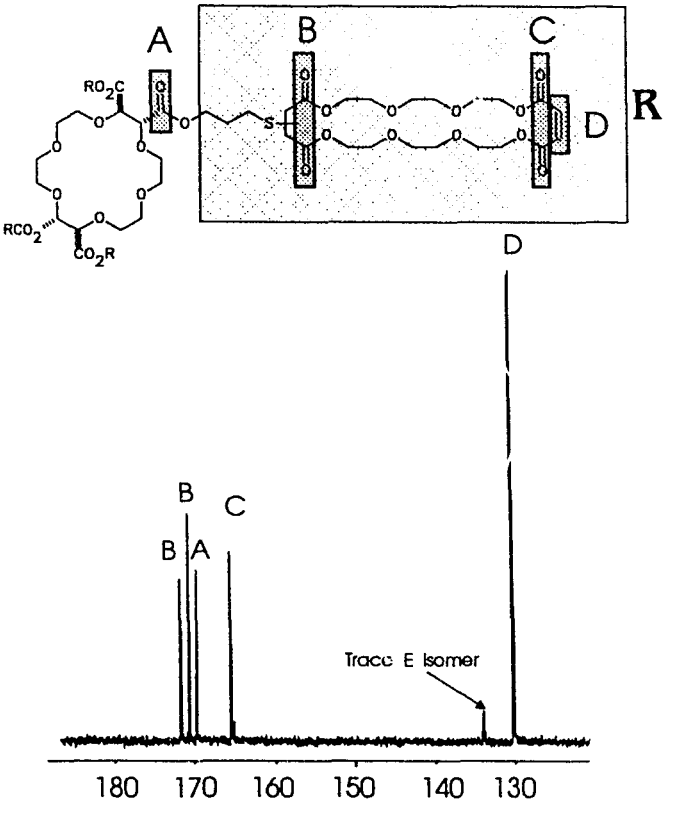


Figure 35. The ^{13}CNMR spectrum for compound **38** as the Z isomer from 190-120ppm.

Table 7. Comparison of the ^{13}CNMR spectrum of **38** with those obtained for compounds **10** and **12** from 190-120ppm.

Assignment	Compound **10**	Compound **12**	Compound **38**
Carbonyl (C)	165.0	165.0	165.4
Carbonyl (B)	-	171.4 170.2	171.5 170.5
Carbonyl (A)	-	-	169.5
Alkene (D)	129.0 (Z)	129.7 (Z)	130.1 (Z) 130.0 (Z)

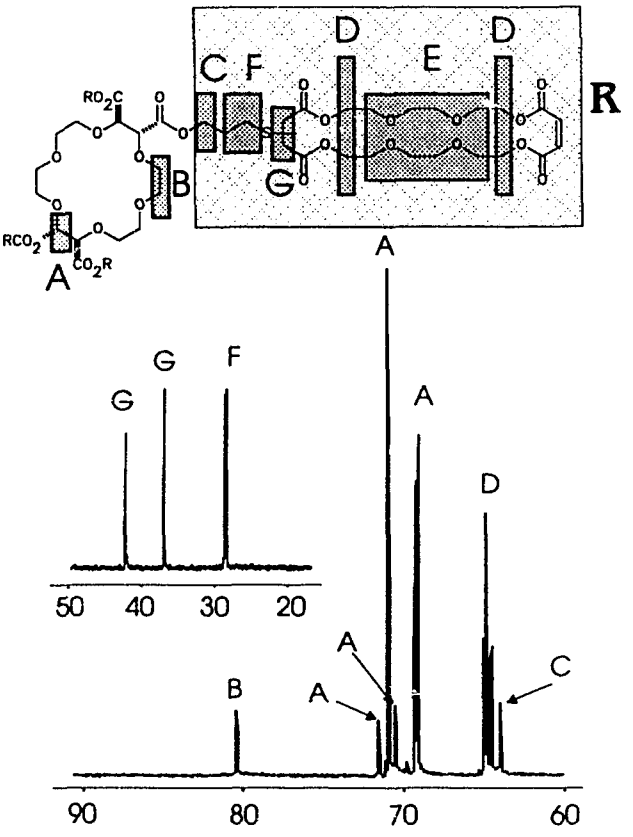


Figure 36. The ^{13}CNMR spectrum for compound **38** as the Z isomer from 90-20ppm.

Table 8. Comparison of the ^{13}CNMR spectrum of **38** with those obtained for compounds **10** and **12** from 90-20ppm.

Table 8 Assignment	Compound **10**	Compound **12**	Compound **38**
Crown CH_2 (A)	-	-	71.5 70.4
Crown CH_1 (B)	-	-	80.2
Macrocycle CH_2O (A)	70.5 68.8	70.5 68.7	70.9 69.2 69.1 69.0
Ester linker CH_2 (C)	-	-	63.9

Table 8 Assignment	Compound **10**	Compound **12**	Compound **38**
Ester Macrocycle (D)	64.3	64.4 64.3 64.0	65.2 64.9 64.8 64.4
Linker CH_2 (F)	-	41.7 36.4	28.5 28.2
CH_2***C***HS (G) ***C***H_2CHS (G)	-	31.8 28.1	42.0 36.8

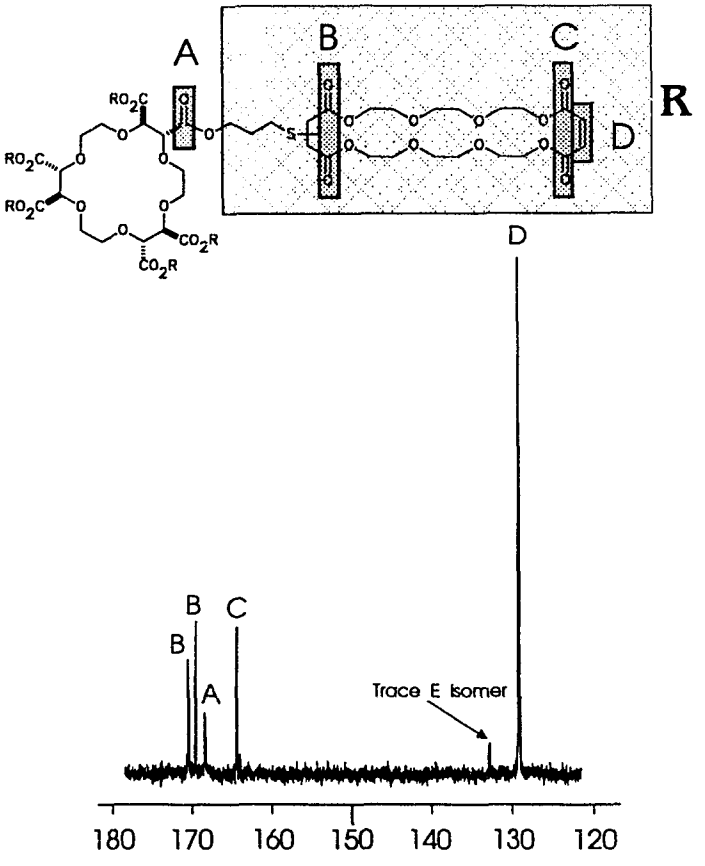


Figure 37. The ^{13}CNMR spectrum for compound **39** as the Z isomer from 190-120ppm.

Table 9. Comparison of the ^{13}CNMR spectrum of **39** with those obtained for compounds **10** and **12** from 190-120ppm.

Assignment	Compound **10**	Compound **12**	Compound **39**
Carbonyl (C)	165.0	165.0	165.5 165.4
Carbonyl (B)	-	171.4 170.2	171.5 170.5
Carbonyl (A)	-	-	169.4
Alkene (D)	129.0 (Z)	129.7 (Z)	130.2 (Z) 130.0 (Z)

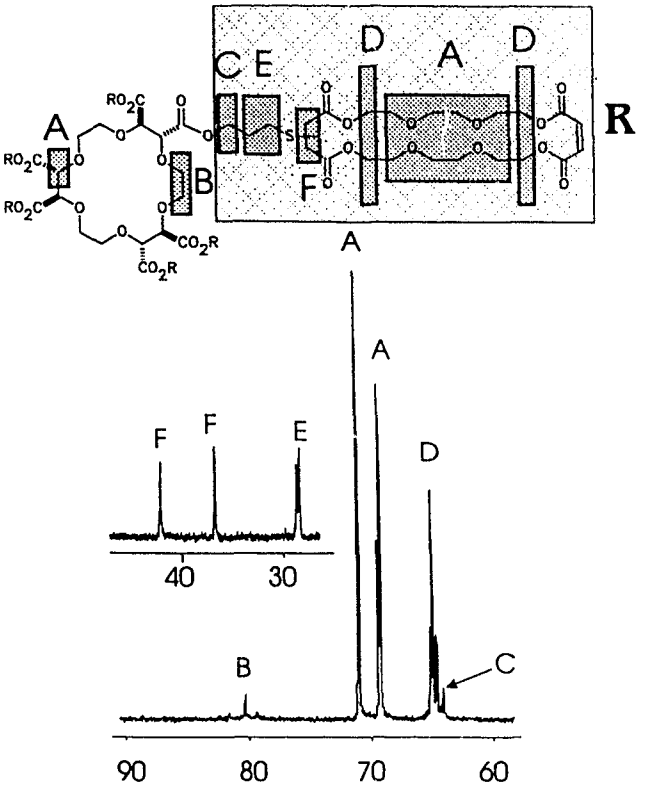


Figure 38. The ^{13}CNMR spectrum for compound **39** as the Z isomer from 90-20ppm.

Table 10. Comparison of the ^{13}CNMR spectrum of **39** with those obtained for compounds **10** and **12** from 90-20ppm.

Table 10 Assignment	Compound **10**	Compound **12**	Compound **39**
Crown CH_2 (A)	-	-	Hidden
Crown CH_1 (B)	-	-	80.3
Macrocycle CH_2O (A)	70.5 68.8	70.5 68.7	70.9 69.2 69.1
Ester linker CH_2 (C)	-	-	63.9

Table 10 Assignment	Compound **10**	Compound **12**	Compound **39**
Ester Macrocycle (D)	64.3	64.4 64.3 64.0	64.9 64.8 64.5 64.2
Linker CH_2 (F)	-	41.7 36.4	28.5 28.3
$CH_{2C}HS$ (G) CH_2CHS (G)	-	31.8 28.1	42.0 36.8

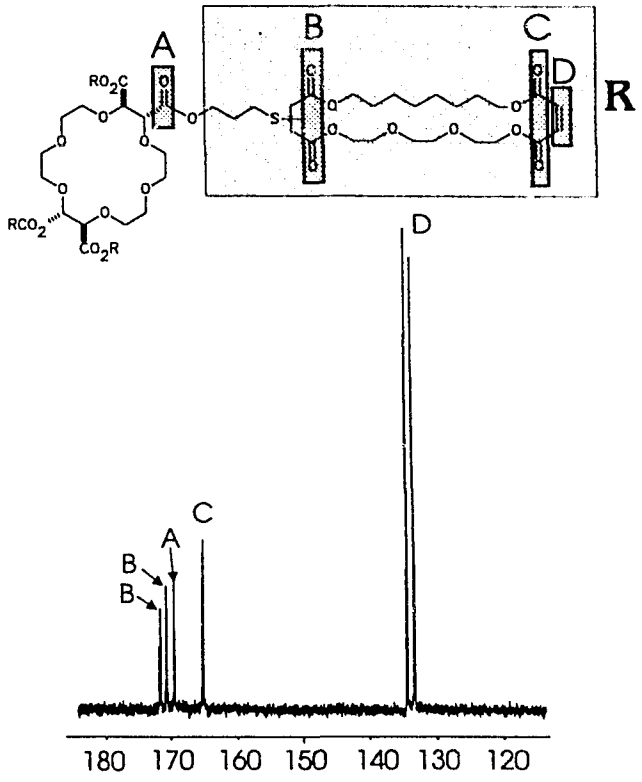


Figure 39. The ^{13}CNMR spectrum for compound **34** as the E isomer from 190-120ppm.

Table 11. Comparison of the ^{13}CNMR spectrum of **34** with those obtained for compounds **7** and **8** from 190-120ppm.

Assignment	Compound **7**	Compound **8**	Compound **34**
Carbonyl (C)	165.1 165.0	164.9	165.2 165.1
Carbonyl (B)	-	171.6 170.5	171.8 171.6 170.8 170.6
Carbonyl (A)	-	-	169.5
Alkene (D)	130.2 (Z) 129.0 (Z)	134.0 (E) 133.1 (E)	134.3 (E) 133.3 (E)

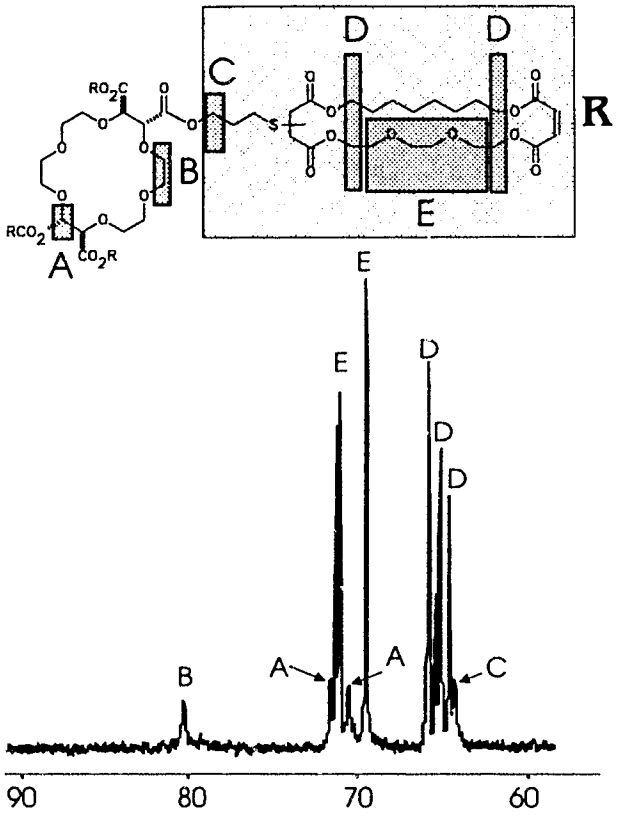


Figure 40. The ^{13}CNMR spectrum for compound **34** as the E isomer from 90-60ppm.

Table 12. Comparison of the ^{13}CNMR spectrum of **34** with those obtained for compounds **7** and **34** from 90-60ppm.

Table 12 Assignment	Compound **7**	Compound **8**	Compound **34**
Crown CH_2 (A)	-	-	71.5 70.5
Crown CH_1 (B)	-	-	80.1

Table 12 Assignment	Compound **7**	Compound **8**	Compound **34**
Macrocycle CH_2O (E)	70.5 68.7	70.7 70.6 70.4 69.2 69.0 68.9 65.3 65.2	71.0 70.8 69.3
Ester linker CH_2 (C)	-	-	64.0
Ester Macrocycle (D)	65.2 64.1	64.7 64.5 64.4 64.2 63.9	65.6 65.5 64.9 64.8 64.3

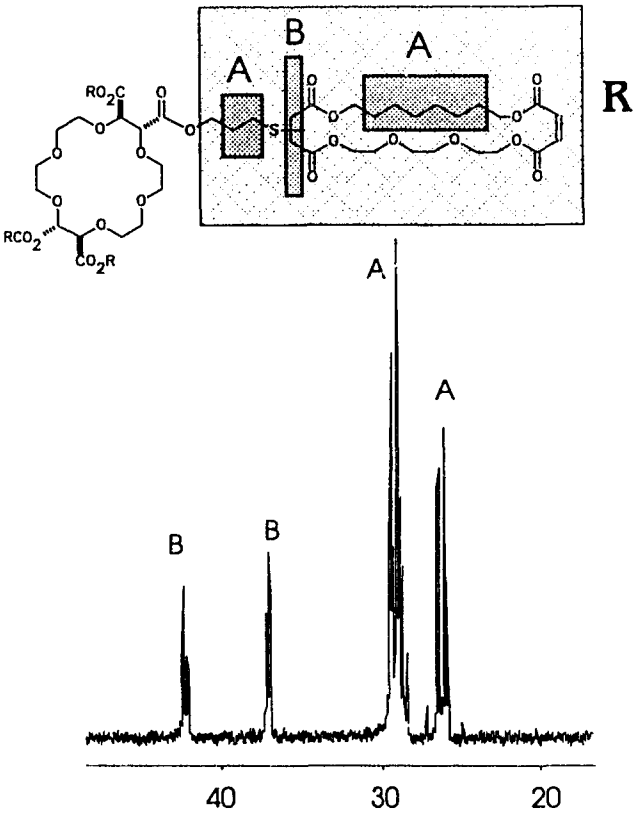


Figure 41. The ^{13}CNMR spectrum for compound **34** as the E isomer from 50-20ppm.

Table 13. Comparison of the ^{13}CNMR spectrum of **34** with those obtained for compounds **7** and **8** from 50-20ppm.

Table 13 Assignment	Compound **7**	Compound **8**	Compound **34**
CH_2**C**HS (B)	-	41.7	42.0
CH_2CHS (B)		41.5	41.8
		36.5	36.9
		36.4	36.7

Table 13 Assignment	Compound **7**	Compound **8**	Compound **34**
Alkyl CH_2 (A)	28.7 28.2 25.5	31.8 31.7 28.8 28.6 28.4 28.3 28.0 25.6 25.3	29.2 29.1 29.0 28.9 28.8 28.7 28.6 28.5 28.3 26.1 25.7 25.6

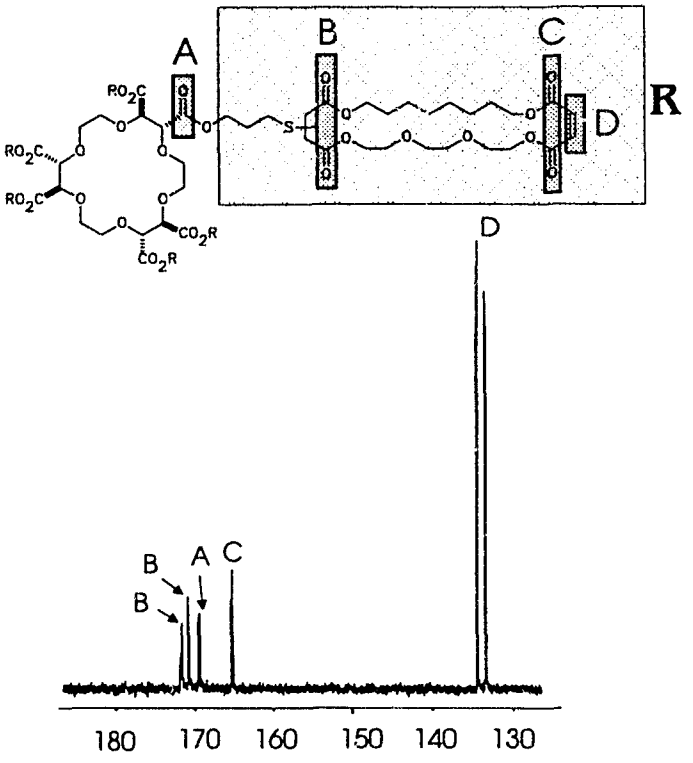


Figure 42. The ^{13}CNMR spectrum for compound **36** as the E isomer from 190-120ppm.

Table 14. Comparison of the ^{13}CNMR spectrum of **36** with those obtained for compounds **7** and **8** from 190-120ppm.

Assignment	Compound **7**	Compound **8**	Compound **36**
Carbonyl (C)	165.1 165.0	164.9	165.2 165.1
Carbonyl (B)	-	171.6 170.5	171.6 170.8
Carbonyl (A)	-	-	169.4
Alkene (D)	130.2 (Z) 129.0 (Z)	134.0 (E) 133.1 (E)	134.3 (E) 133.3 (E)

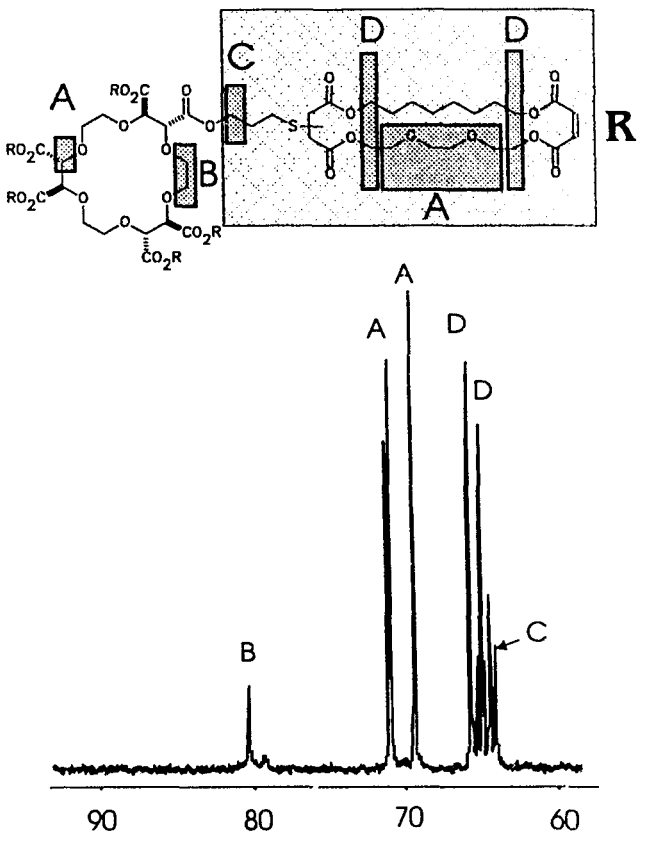


Figure 43. The ^{13}CNMR spectrum for compound **36** as the E isomer from 90-60ppm.

Table 15. Comparison of the ^{13}CNMR spectrum of **36** with those obtained for compounds **7** and **8** from 90-60ppm.

Table 15 Assignment	Compound **7**	Compound **8**	Compound **36**
Crown CH_2 (A)	-	-	Hidden
Crown CH_1 (B)	-	-	80.2

Table 15 Assignment	Compound **7**	Compound **8**	Compound **36**
Macrocycle CH_2O (E)	70.5 68.7	70.7 70.6 70.4 69.2 69.0 68.9 65.3 65.2	71.1 71.0 70.8 69.3 69.2
Ester linker CH_2 (C)	-	-	63.9
Ester Macrocycle (D)	65.2 64.1	64.7 64.5 64.4 64.2 63.9	65.6 65.5 65.1 64.9 64.8

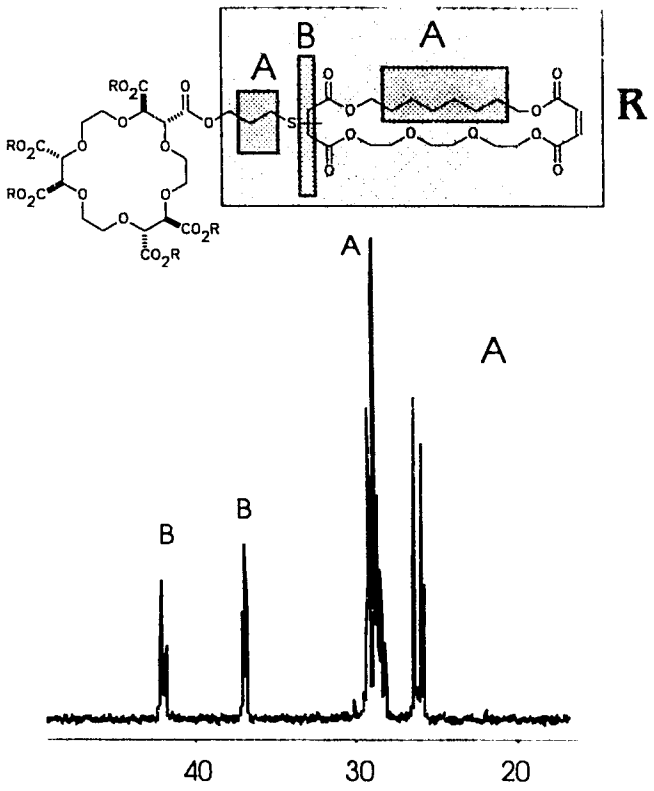


Figure 44. The ^{13}CNMR spectrum for compound **36** as the E isomer from 50-20ppm.

Table 16. Comparison of the ^{13}CNMR spectrum of **36** with those obtained for compounds **7** and **8** from 50-20ppm.

Table 16 Assignment	Compound **7**	Compound **8**	Compound **36**
CH_2**C**HS (B)	-	41.7	42.0
CH_2CHS (B)		41.5	41.7
		36.5	36.9
		36.4	36.7

Table 16 Assignment	Compound **7**	Compound **8**	Compound **36**
Alkyl CH_2 (A)	28.7 28.2 25.5	31.8 31.7 28.8 28.6 28.4 28.3 28.0 25.6 25.3	29.2 29.0 28.9 28.7 28.6 28.5 28.3 28.0 26.2 25.8 25.6

There are many choices for thio linked head groups. The four representatives chosen were: β-D-1-mercaptoglucose (bulky/neutral), 3-mercaptopropanol (neutral), thioacetate (negative) and 2-mercaptoethylamine hydrochloride (positive).

The addition of β-D-1-mercaptoglucose was carried out in a 50/50 mixture of THF/(isopropanol) at 50°C for 12 hours, with both methane sulphonic acid and 2,2,6,6-tetramethylpiperidine used to adjust the pH to 8. 2,2,6,6-Tetramethylpiperidine was used to avoid competing base addition. The addition of 3-mercaptopropanol was carried out in IPA at 50°C for 3 hours with 2,2,6,6-tetramethylpiperidine as the base catalyst. The addition of thioacetic acid was carried out in THF at 50°C for 3 hours, with 2,2,6,6-tetramethylpiperidine added to adjust the pH to 8 and act as a base catalyst. All of the channel mimics with either thioglucose, thiopropanol or thioacetic acid head groups possess characteristic ^{13}CNMR signals associated with the crown methine (*C*HO) at ~80 ppm, alkyl ester carbon of the linker (CO_2CH_2) at ~65.3 ppm, thioether methine carbon ($SCHCH_2$) at ~42 ppm and the macrocycle carbon adjacent to the linker ($SCHCH_2$) at ~37 ppm. All compounds display an absence of alkene in either the ^{1}HNMR or ^{13}CNMR spectra. The thiopropanol adducts are characterised by hydroxymethyl carbon signals (CH_2OH) at ~61 ppm. The glucose adduct shows characteristic signals in the ^{13}CNMR corresponding to two possible diasterioisomeric linkages with the macrocycle. In particular the anomeric carbon is observed as two signals

at ~86 and ~85 ppm. The doubling of the carbon signals associated with the 1-thioglucose adduct are not the result of isomerisation of the β-anomer to the α-anomer under the reaction conditions. The above premise is confirmed by:

1. Comparison of final product spectra with the simpler spectra associated with the reaction of 1212 with 1-thioglucose. This product has been characterised by Fuhrhop[31] as the addition product of the β-anomer.
2. The ^{13}CNMR spectral complexity is lower than expected for both anomers present. Rather than two signals as were observed for the anomeric carbon, four signals would be predicted for the four diastereomers of the (Macrocycle)R_2*C*H-S-*C*HR_2(Glucose) unit.

The most convincing evidence would be the coupling between the protons of C1 and C2 in the glucose unit. The diaxial disposition of the protons for the β-anomer would result in a larger coupling constant (~12Hz). Sadly, these signals are obscured in the complex ^{1}HNMR spectra of the final products.

The addition of 2-mercaptoethylamine was carried out under analogous conditions but degradation products were isolated, due to the nucleophilic attack of the primary amine on the ester linkage of the crown ether. The macrocycle esters of the wall units are not attacked since the addition of 2-mercaptoethylamine to compound **2** results in isolation of the addition product with no trace of hydrolysis or degradation.

Therefore in the final suite there are three possible head group

combinations. Coupling with the twenty five possible core/wall products means seventy five possible channel mimics exist. The final set prepared is a representative twenty one compounds. (**Figure 45**)

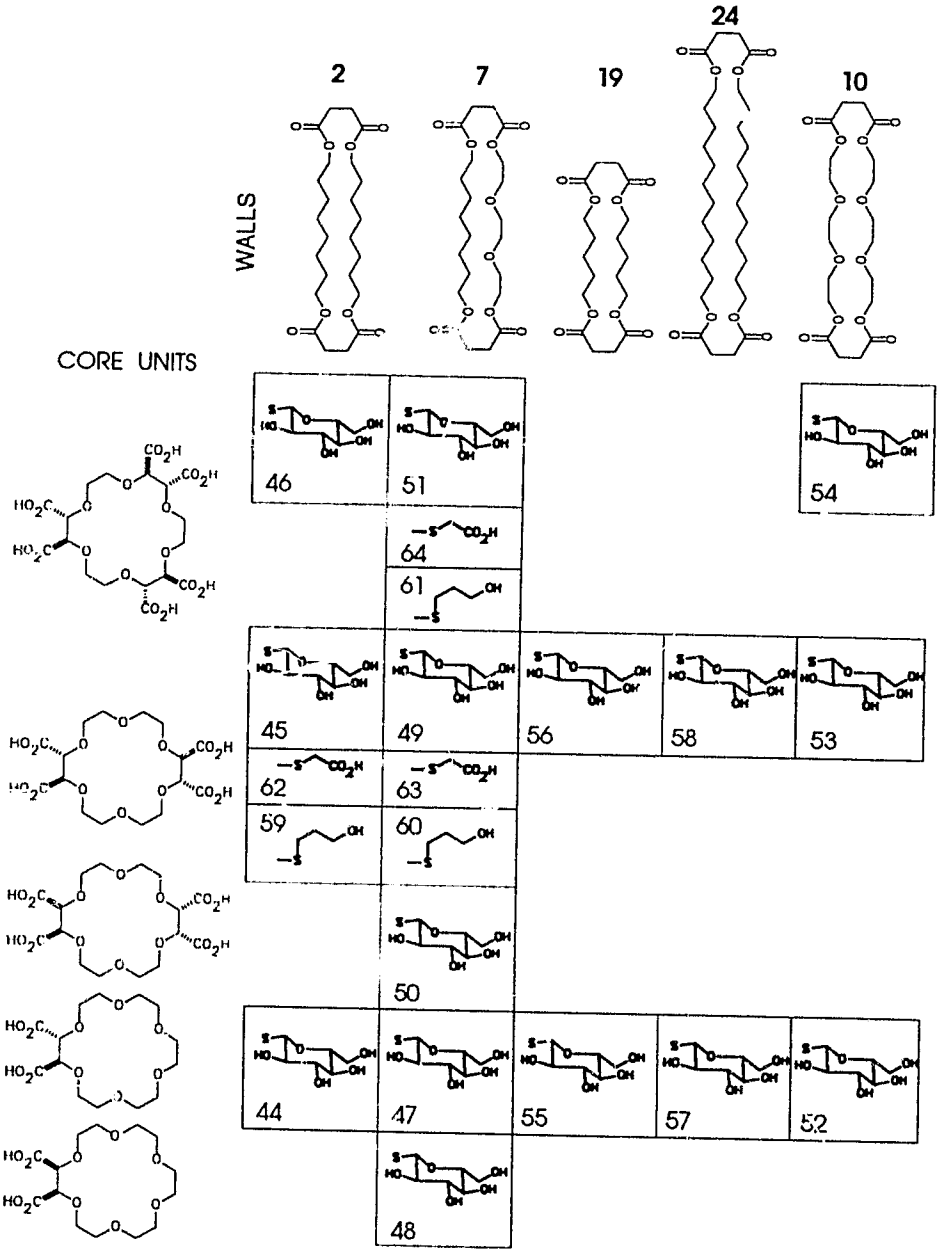


Figure 45. The twenty one molecules synthesised.

TRANSPORT

PROCEDURES

The experimental methods for this section can be found in Appendix 1, as adapted from the M.Sc. Thesis of K. C. Kaye.

The method used for the preparation of egg L-α-lecithin vesicles was the widely employed method of reverse evaporation[42]. This procedure results in large unilameller vesicles (LUV) with good reproducibility in vesicle morphology and monodispersity. The pH-stat experiment[43-46] requires vesicles with high entrapment efficiency which are stable with respect to the time frame of the experiment. Initial investigations into the preparation of such vesicle systems were carried out by Dutton and myself. The quality and reproducibility of this system was subsequently improved by Kaye. Much of the work in this area is still a "black art". The reason for failure in a preparation is not always clear; however the system as developed by Kaye is consistently reproducible.

The method as employed by Kaye starts with the addition of aqueous buffer to a solution of lipid in ether. This two phase system is then sonicated using a probe source to cause micelle formation which is followed visually by metamorphosis through a cloudy and finally to a translucent/opaque single phase. This opalescent solution is then evaporated under reduced pressure at ca. 25°C with slow rotation of the sample. All the ether must be removed but little or no water must be removed in this step, so a close eye must be kept on

the system during this operation. The removal of ether from the system is observed by the formation of large bubbles. This occurs for approximately 10 minutes. The solution then congeals into a thick "marmalade" phase which slowly evolves bubbles for a further 15 minutes and then rapidly collapses into a mobile aqueous phase. This is the point at which the vesicles are formed. An aliquot of the internal buffer is added and the system is evaporated under reduced pressure a further 30 minutes to remove the remaining traces of ether. Sizing of the vesicles is carried out by forcing the solution through successive polycarbonate membranes. The internal buffer present on the exterior of the vesicle membrane is then removed by passing the solution down a short, pre-equilibrated Sephadex G25 disposable column with external solution.

The above procedure results in a solution that contains vesicles with an average diameter of 1200Å as determined from transmission electron micrographs. About 5% of the vesicles were between 2500 and 3500Å, and a further 5% were less than 600Å in diameter. The solution also appears to contain large lipid aggregates. The vesicle solution's quality or percent of unilameller vesicles was ascertained by a standard melittin assay[47]. Melittin is present as an active component of bee venom and can only rupture one lamella of a vesicle since melittin enters the lipid irreversibly. This process can be thought of like the pealing of an onion by removal of one layer at a time. The assay depends on sufficient melittin added to remove all the external layers with a single addition. Incremental addition of melittin results

in the removal of the second and subsequent layers, each layer becoming successively the exterior layer. For our vesicle solutions, 95% of the entrapped buffer was present in unilameller vesicles. This implies that the aggregates observed by electron microscopy contain very little entrapped buffer. These aggregates should not be neglected however since the compounds tested will partition into this lipid as well as the desired LUV. Partition into the aggregates will be an unobserved event since these aggregates contain no titrable protons.

For a typical pH-stat experiment about 0.2mL of vesicle solution was used which corresponds to approximately 1.9mg of phospholipid. This volume of vesicle solution results in entrapped protons requiring between 0.3 and 0.5mL of choline hydroxide solution to neutralise. For this particular assay Triton X100 was used since this detergent ruptures all structures (whether or not the structures are unilameller or multilameller) to release the total titrable protons. Protocols established by Kaye ensure that a typical transport experiment results in collection of more than 100 data points.

For the above assays and subsequent transport evaluation experiments the titration cell contains 4mL, the remaining 3.8mL coming from the external solution (choline sulphate and D-mannitol). The observed pH (about 5.6) is a result of a combination of the external and vesicle solutions. The bis-Tris buffer used as the external solution was prepared at pH 6.6. In order to establish a unity pH gradient across the vesicle membrane, the end-point was

set to 7.6. Choline hydroxide was added to achieve this gradient; the volume of base required was typically 1mL or less. A volume of greater than 1mL either implies breakdown of the vesicles during the preparation or inefficient removal of internal buffer by the gel column. Once the gradient has been established, this is the start of the experiment and data is collected. A cartoon of the experiment is given in **Figure 46** and a typical output is given in **Figure 47**.

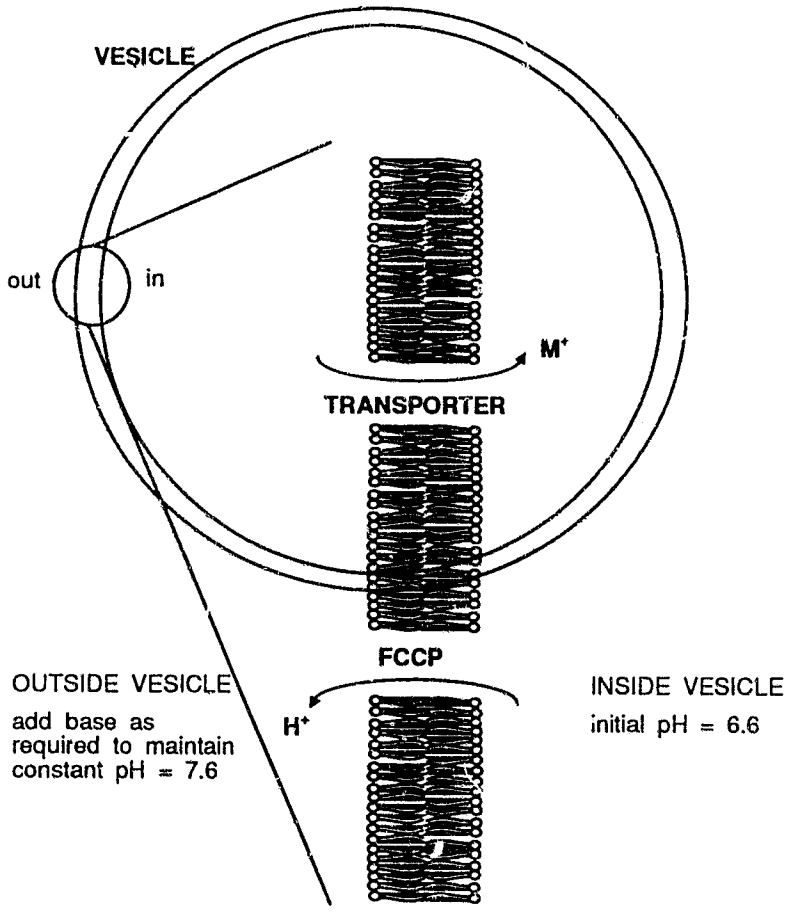


Figure 46. Schematic of cation and proton antiport through an ion channel in a pH-stat experiment.

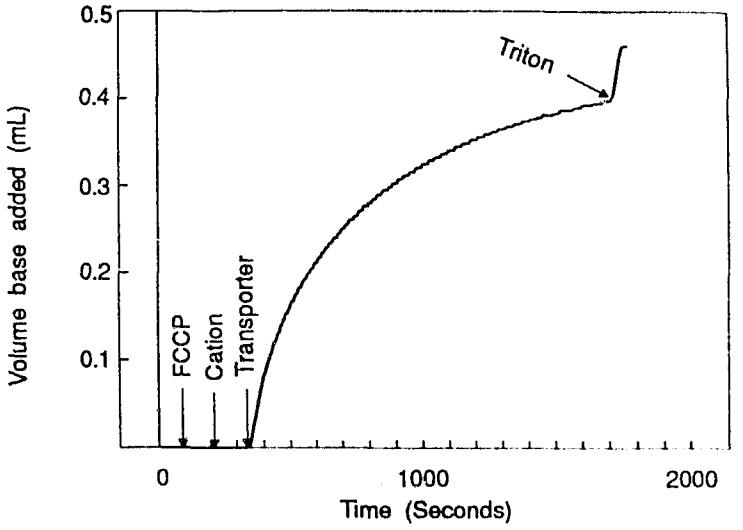


Figure 47. Typical pH-stat experiment showing plateau behaviour of titrant volume added *versus* time elapsed. Compound **49** at a concentration of 0.62 μM and giving a rate of 31×10^{-10} mol H^+s^{-1}

The first additive to the system is the proton carrier FCCP[48], added as a methanol solution. This compound is added to ensure electroneutrality of the transport. The transporter may be unable to facilitate the transport of protons. It is also added to ensure that transport is not limited by the rate of proton flux. It should be noted that for very active systems this assumption may break down. For all the transport experiments, a volume of FCCP was added such that the initial concentration within the pH-stat cell was between 0.15 and 0.18μM. This amount of FCCP induces a very slow base rate of leakage in the presence of metal ions in the absence of a transporter, but the rate is typically less than 1×10^{-10} mol H^+s^{-1}.

For the usual transport experiment the metal ion is the next additive. Commercially available alkali metal sulphate salts at a concentration of 0.500

M (with no pH adjustment) are employed. This order of addition is mainly for operational convenience. Upon addition of metal ion, the cell solution pH drops below 7.6. For lithium and cæsium this effect was small, but with sodium and potassium this effect was larger, and rubidium was observed to have the largest effect. The lowering of the pH was not caused by antiport of metal ion and protons across the membrane, neither was it due to vesicle lysis. If the original pH of 7.6 is reestablished and the system then lysed using Triton, the same lyse volume for this and the system without metal is found. For a typical experiment the metal ion is added with the titrimeter turned off. The pH then drops and becomes stable, the titrimeter is then turned on and the set pH (7.6) is reestablished by addition of base. Transporter is then added and transport is observed as a volume of added base as a function of time.

Conversely, when the transporter is added as the second additive and the metal ion is added subsequently, the pH drops below 7.6 as discussed above. The titrimeter is turned off initially to allow for equilibration within the system. The set pH is adjusted to whatever pH is established and the titrimeter is activated again. Transport is observed but now there is no consistency of the final pH between runs. The time allowed by the operator before restarting the titrimeter will also affect the experiment by determining the final pH. Both these factors make this method undesirable.

RESULTS

The complete data set I obtained from investigations of the 21 compounds prepared and discussed within the synthesis section is given in Table 23 (pages 107-116).

In most cases the transport experiment appears as depicted in **Figure 47**, The curve can be analysed using a first order analysis of the data up to the observed plateau. For low transporter concentrations this is less than 95% of the total titrable protons. The 95% level corresponds to the total LUV ation as determined by the melittin assay. The remaining 5% nds to multilameller vesicles that remain unaffected by the transporter entration.

amicidin and amphotericin were studied by Herve *et al.*[45] in a similar vesicle system to that employed for this study. In their analysis Herve *et al.*[45] assume that two populations of vesicles exist. The first population contains active transporters and the second contains no active transporters. From studies in soybean phospholipid LUV's, the transport rate for gramicidin is known to be rapid. It takes less than one second to totally equilibrate each vesicle[49]. From single channel conductance measurements, a typical conductance for gramicidin is 3 picosiemens, indicating a very rapid transfer of metal ions across the black lipid membrane (BLM)[1]. The observed rate process in the pH-stat experiment is much slower, ion transport cannot be

the process observed in the experiment. Using ^{31}PNMR, Herve *et al.*[45] observed two different populations corresponding to equilibrated and non-equilibrated vesicles. The two populations become constant after a time comparable to the time to attain the plateau in the pH-stat experiment. Only after long periods of time do the populations equilibrate. This result indicates that gramicidin moves slowly between vesicles. The observed rate of the pH-stat experiment is then due to some initiation or gating of the gramicidin channel within the lipid bilayer. If an inactive form converts to an active form, rapid ion transport could occur and the vesicle would be equilibrated within the duration of the channel opening. This mechanism is consistent with the observed first order curve. The instantaneous rate is proportional to the remaining population of vesicles containing gramicidin since the gating event is assumed to be stochastic. Note as well that the rate of transport is proportional to the extent of transport.

With valinomycin the concentration dependant plateau behaviour was <u>not</u> observed[45]. The same plateau is achieved at <u>all</u> concentrations. From ^{31}PNMR investigations of valinomycin in vesicle systems, only one population of vesicles exist at any time. Valinomycin is therefore simultaneously affecting the total population of vesicles. Expressed kinetically, the rate valinomycin moves between vesicles is fast (on the NMR time scale) and the observed rate process is due to slower metal ion transport. Unlike gramicidin, the plateau is independent of rate or transporter concentration. Rate is, however,

proportional to transporter concentration.

My systems show a variety of relationships for the rate, extent of transport and transporter concentration between these two extremes.

For compound **51** the relationship is similar to that observed by Herve *et al.*[45] for gramicidin. (**Figure 48**) From the figure it can be seen that rate and extent of transport are proportional to concentration. This particular set of plots contains only three data points. More data points were not collected for this compound since Kaye studied this compound in depth[50]. The large data set obtained by Kaye for **51** displays a similar concentration dependence for the rate and extent of transport.

The extents of transport used are based on the extents calculated directly from the experimental data. The error bars for this quantity are large since in many cases the observed transport event was only monitored for three half lives. These error bars indicate a maximum spread that the extent of transport can take, without producing a significant deviation in the calculated first order curve from the observed first order curve. It should be noted that the error in extent of transport at lower extents of transport is less significant. However these large variations in the extent of transport have a small effect on the calculated rates (less than the 10% variation observed between multiple runs).

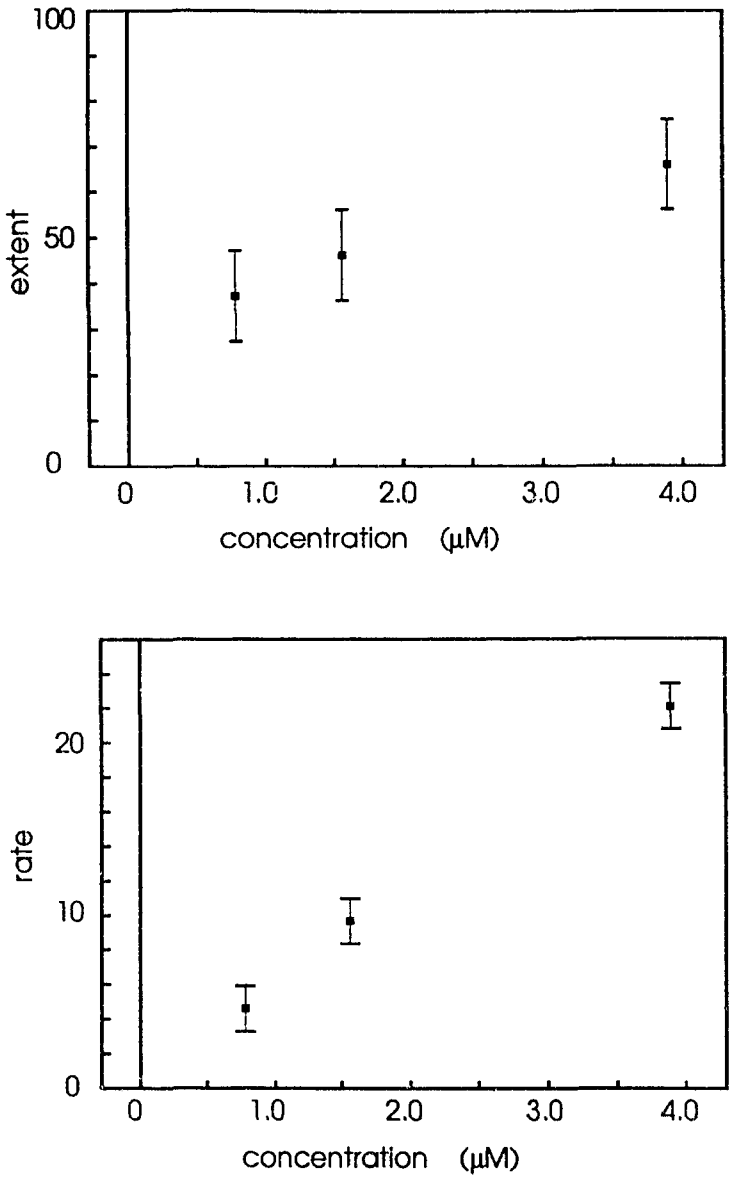


Figure 48. Top: extent of transport as a function concentration for compound **51**. Bottom: rate of transport mol H^+ s^{-1} ($x10^{10}$) for compound **51** as a function of concentration. refer to Table 23 (pages 107-116) for details.

The relationship of rate and extent of transport as a function of concentration observed for compound **50** are similar to that observed for valinomycin by Herve *et al.*[45]. (**Figure 49**) Here the extent is independent of concentration, but the rate is proportional to the concentration of transporter.

Compound **44** lies somewhat between these two extremes. (**Figure 50**) For low concentrations of transporter both the rate of transport and extent of transport is proportional to concentration. However, at high concentrations the extent of transport is constant at close to the MLV limit.

Compound **49** is an even more complex case. (**Figure 51**) Here the rate of transport concentration dependence is biphasic. For each region the rate is proportional to the concentration of transporter. In the low concentration region, the extent of transport and the rate of transport are both proportional to concentration. At higher concentrations the rate of transport is still dependent but the extent of transport is independent of concentration. Note that a break occurs at about 0.48μM in the extent of transport and at about 0.6μM in the rate of transport plots.

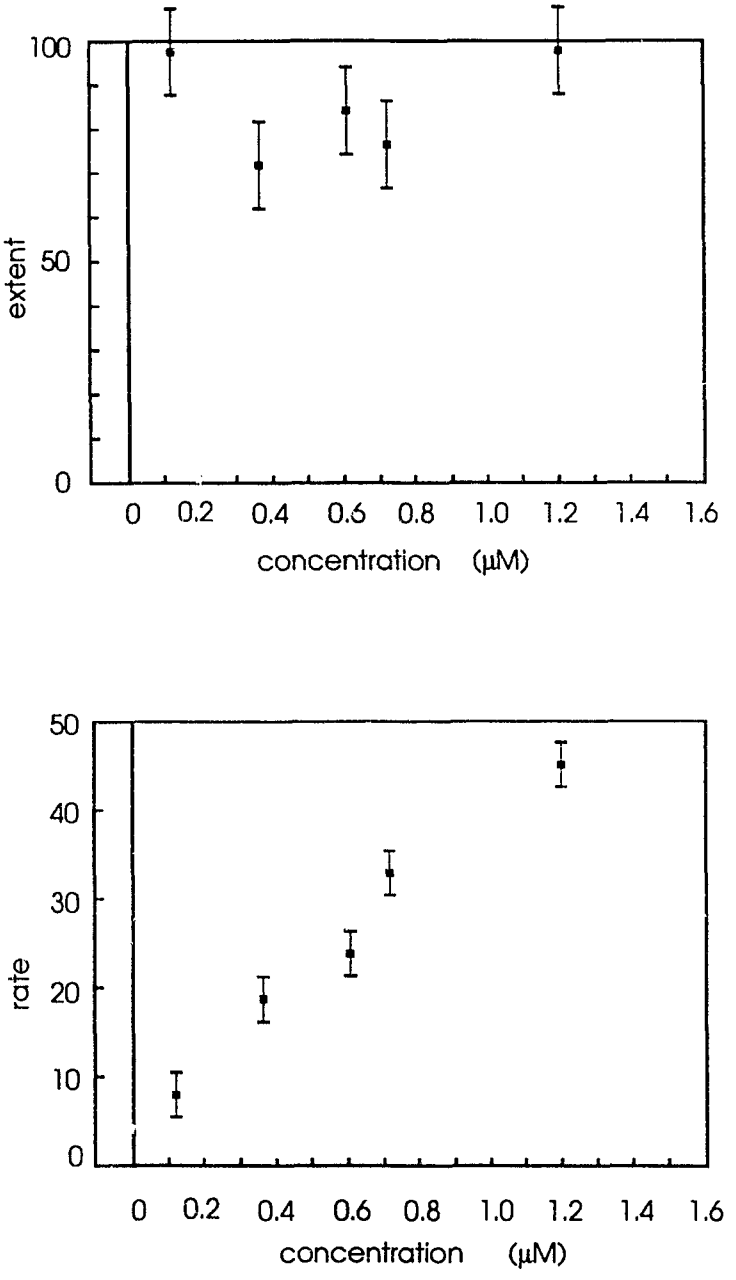


Figure 49. Top: extent of transport as a function of concentration for compound **50**. Bottom: rate of transport mol H^+ s^{-1} ($x10^{10}$) for compound **50**. Refer to Table 23 (pages 107-116) for details.

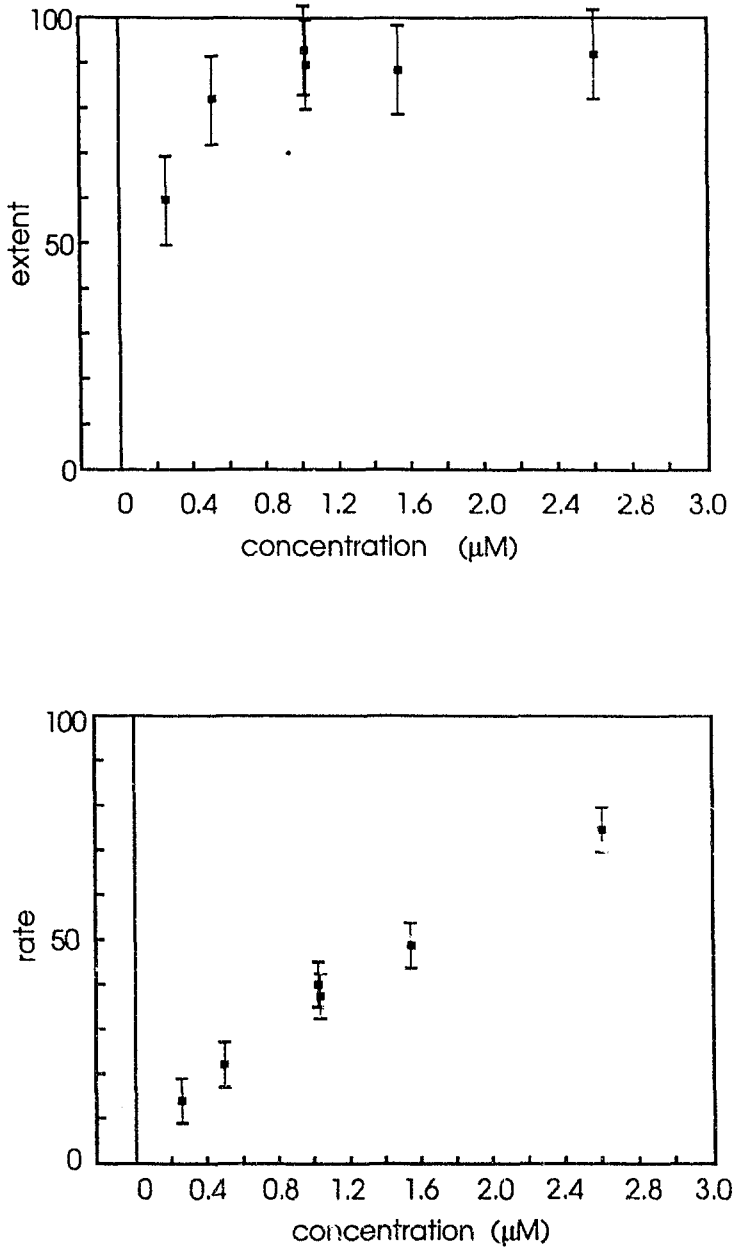


Figure 50. Top: extent of transport for compound **44** as a function of concentration. Bottom: rate of transport mol H^+ s^{-1} ($x10^{10}$) for compound **44** as a function of concentration. Refer to Table 23 (pages 107-116) for details

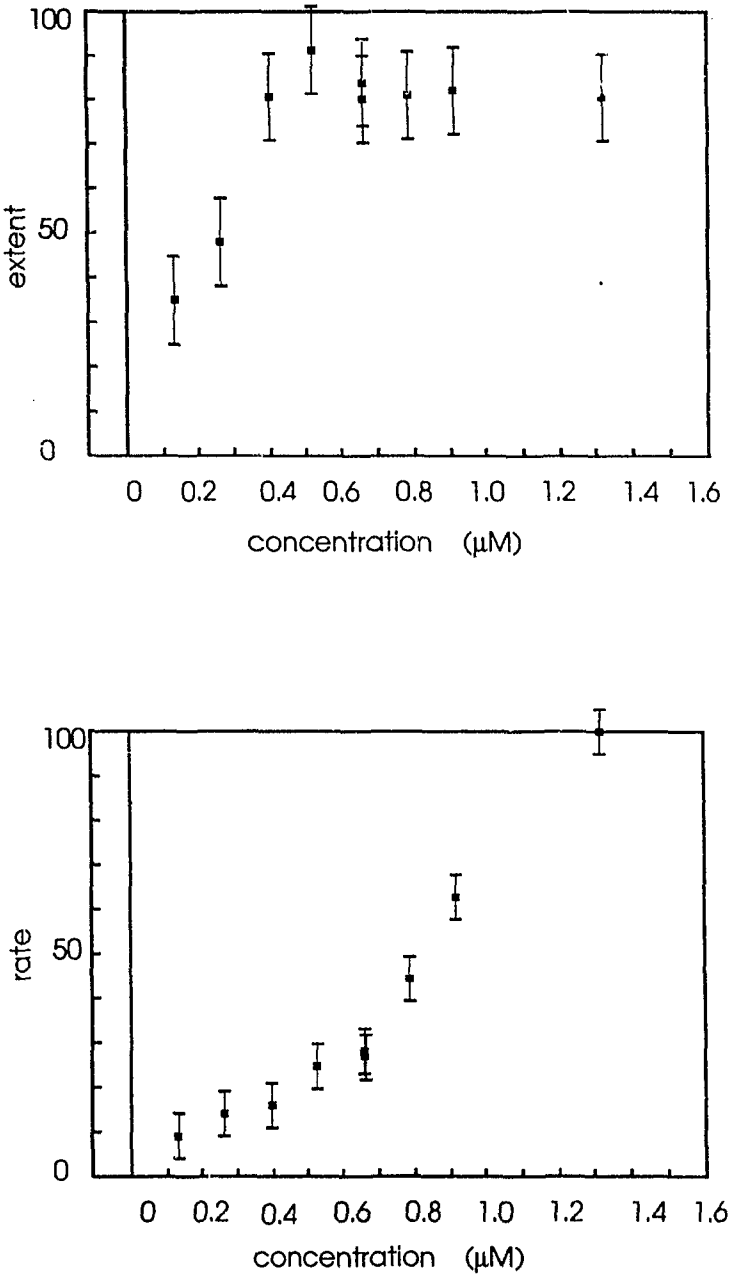


Figure 51. Top: extent of transport for compound **49** as a function of concentration. Bottom: rate of transport mol H^+ s^{-1} ($x10^{10}$) for compound **49** as a function of concentration. For details refer to Table 23 (pages 107-116).

In some cases the transport rate behaves in a zero order manner as depicted. (**Figure 52**) This behaviour is very interesting since it implies proton release is independent of the fraction of entrapped protons in the remaining intact vesicles. In this case the extent of transport is somewhat meaningless since in all cases the 95% MLV is eventually reached. The compounds that display this behaviour are **45, 62** and **46**.

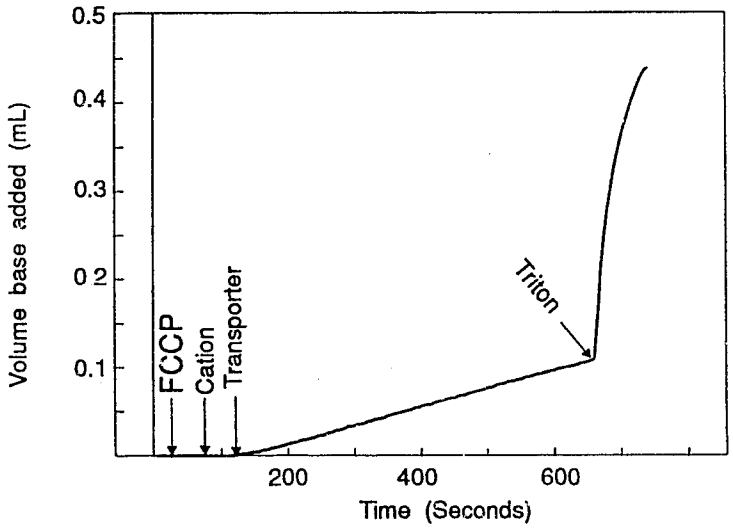


Figure 52. Typical zero order pH-stat experiment of titrant volume added *versus* time elapsed. Compound **45** at a concentration of 0.32μM and giving a rate of 8.2×10^{-10} mol H^+ s^{-1}.

The rate constants for all transport events were calculated according to either first or zero order analysis as appropriate from the experiment. The absolute rate of moles of protons released per second was calculated by multiplying the observed rate constant which is a function of volume, by the concentration of the base used. The raw data is reproducible between different batches of vesicles only to the extent of ±50%. The reproducibility between

experimental runs within the same batch is much better and at its worst is only ±10%. The error between days can be compensated by normalising with respect to a particular compound and concentration. With this work, I have chosen not to do this manipulation even though it is possible. All rates used will represent the raw data without any manipulation. All the important qualitative comparisons are larger than the batch to batch variations and the important quantitative comparisons were carried out using the same vesicle solution.

For qualitative comparisons between transporters day to day comparison must be made. The table below categorises the compounds by their activity. In this case activity is taken as the observed rate divided by the concentration of the compound (μM). Only the most active value for each compound observed with potassium sulphate at 98mM concentration is given in the table. Recall that differences greater than 50% are significant.

Table 17. Activities of the mimics; rate per unit concentration.

Table 17 Mimic[5] **T**	Batch[1]	[T][7]	Rate[2]	Rate /[T]	Relative Activity[3]
		μM	$1x10^{-10}$	$1x10^{-4}$	
57	5	3.0	1.0	0.33	0.085
59	4	2.4	1.0	0.42	0.11

Table 17 Mimic[5] **T**	Batch[1]	[T][7] µM	Rate[2] $1x10^{-10}$	Rate /[T] $1x10^{-4}$	Relative Activity[3]
53	4	2.2	1.0	0.46	0.12
61	2	3.1	2.8	0.90	0.23
58	4	3.1	3.2	1.0	0.26
64	10	3.2	4.1[4]	1.3	0.33
54	4	3.5	5.0	1.4	0.36
52	5	2.2	4.6	2.1	0.54
60	2	2.3	6.4	2.7	0.69
46	4	2.0	7.9[4]	3.9	1
56	4	1.3	7.8	6.2	1.6
55	5	3.3	22.	6.8	1.7
51	2	0.76	6.3	8.2	2.1
48	5	2.8	27.	9.6	2.5
63	2	0.51	6.0	11.7	3.0
47	7	3.0	36.	12.	3.1

Table 17 Mimic[5] **T**	Batch[1]	[T][7] µM	Rate[2] $1x10^{-10}$	Rate /[T] $1x10^{-4}$	Relative Activity[3]
62	8	2.0	27.[4]	13.	3.3
45	4	0.32	8.2[4]	25.	6.4
44	7	0.26	17.	64.	16
49	3	1.3	100.	79.	20
50	6	0.57	48.	83.	21
Gram[6]	5	0.025	66.	2700.	690

1)Indicates the batch number for the vesicles used in the pH-stat experiment.
2)Rates calculated as a first order process, unless otherwise noted. Units: mol H^+ s^{-1}.
3)Activities relative to compound **46**
4)Rates calculated as a zero order process. Units mol H^+ s^{-1}.
5)FCCP concentration: 0.15-0.18µM.
6)Gramicidin
7)Concentration of mimic

If an activity (expressed as rate per unit concentration) of less than $6x10^{-4}$ mol H^+ s^{-1} per mol of mimic is taken arbitrarily to indicate an inactive system then compound **46** is on the borderline (this compound is within the 50% allowed variation) all other compounds have their activities expressed relative to this compound. From this analysis nine inactive compounds are designated; these compounds are: **57**, **59**, **53**, **61**, **58**, **64**, **54**, **52** and **60**.

For some of the active compounds an "add-back" experiment was carried

out. This add-back experiment is similar to the normal experiment except 2.0mL of the experimental solution is removed prior to transporter addition. The transporter is then added to the remaining solution as in the typical experiment. Once the transport event has finished, the aliquot that was removed is returned to the titrimeter cell and the transport monitored further. (**Figure 53**) This experiment determines the facility of a transporter's movement between vesicles. If a transporter is very "promiscuous" then the rate in the second region will be high; conversely, the second rate will be low if the transporter remains in the vesicle it first enters. The maximum rate can only be about half that observed for the first event since the concentration of transporter is halved after add back. The result shown below is for a molecule with a high degree of mobility between vesicles. The results for this system and three other compounds are given in **Table 18**.

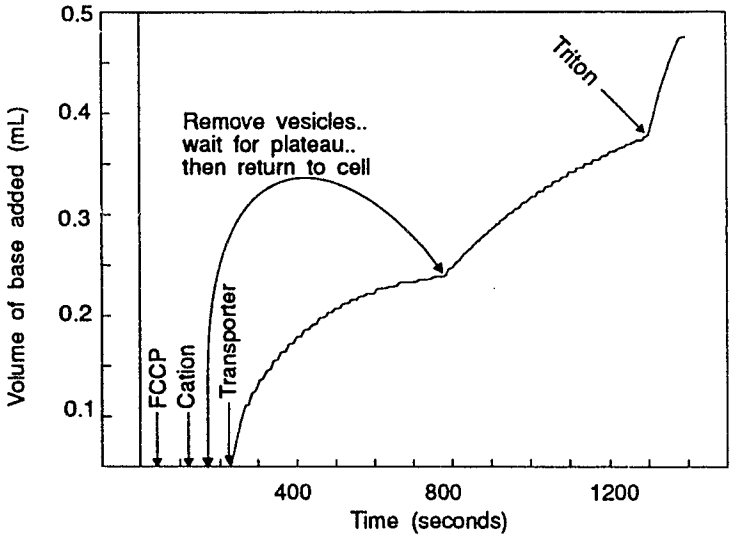


Figure 53. Typical pH-stat (add back) experiment of titrant volume added versus time elapsed (compound **49**)

Table 18. The ability of the transporters to migrate between vesicles as determined by pH stat (ADD BACK) experiments.

Mimic[3]	Rate[1] before add back	Rate[1] after add back
	$1x10^{-10}$	$1x10^{-10}$
51	48	12
49	27	10
44	42	18
62	16^{2}	9^{2}

1)Rates calculated as a first order process, unless otherwise noted. Units: mol H^+ s^{-1}.
2)Rates calculated as a zero order process. Units mol H^+ s^{-1}.
3)FCCP concentrations used 0.15-0.18μM.

A typical evaluation study on transporters involves a study of the concentration dependence of both the transporter and metal ion. The apparent kinetic order of some of the transporters using the data from Table 23 (pages 107-116) was calculated by the method of initial rates:

$$\frac{d[A]}{dt} = k_{obs}[A]^a[B]^b..[N]^\pi$$

$$\log\frac{d[A]}{dt} = \log k_{obs} + a\log[A] + ...n\log[N]$$

From a graph of log (initial rate) versus log[A], the slope gives the apparent kinetic order for the A component. Table 19 shows the calculated

apparent orders for **62**, **45**, **44**, **50**, **63**, gramicidin and valinomycin.

Table 19. The apparent order of some transporters as determined from the initial rates of transport.

Mimic	Apparent Order in [T]
62	0.20±0.07 (r^2=0.90)
45	0.43±0.02 (r^2=0.99)
44	0.66±0.04 (r^2=0.99)
63	0.76±0.09 (r^2=0.99)
50	0.75±0.05 (r^2=0.99)
51[1]	0.45±0.07 (r^2=0.92)
Gramicidin[1]	0.39±0.07 (r^2=0.96)
Valinomycin[1]	0.50±0.07 (r^2=0.98)

1)These particular values come from the Masters thesis of K. C. Kaye[50].

Compound **49** was not included in this table because it has biphasic behaviour which is depicted in the concentration versus rate profile given (**Figure 51**). At low concentrations of transporter the apparent order is calculated to be 0.69±0.07(r^2=0.96 for 6 data points) at higher concentrations the apparent order is 2.0±0.2 (r^2=0.97 for 5 data points).

The potassium ion concentration dependence of transport for **47**, **49**, gramicidin and valinomycin was studied. The observed saturation can be analysed in terms of Michaelis-Menton parameters V_{max} (the maximum rate of transport) and K_m (the concentration of cation to obtain half the maximum rate).

A Lineweaver-Burk analysis was used:

$$\frac{1}{\langle \frac{d[X]}{dt} \rangle} = \frac{1}{[M^+]} \left(\frac{K_m}{V_{max}} \right) + \frac{1}{V_{max}}$$

A plot of 1/rate versus $1/[M^+]$ gives an intercept of $(V_{max})^{-1}$, and a slope multiplied by V_{max} gives K_m.

A Eadie-Hofstee analysis is also possible:

$$\frac{d[M^+]}{dt} = V_{max} - K_m \frac{d[M^+]}{dt} [M^+]$$

A plot of rate versus rate / $[M^+]$ gives an intercept of V_{max} on the y-axis, and a slope of $-K_m$.

These analyses were carried out on two compounds and the calculated values for V_{max} and K_m are given in Table 20.

Table 20. Michaelis-Menton parameters V_{max} and K_m, determined by Lineweaver-Burk and Eadie-Hofstee analyses of potassium ion concentration dependence of selected transporters.

Mimic	Lineweaver-Burk		Eadie-Hofstee	
	V_m[1]	K_m[2]	V_m[1]	K_m[2]
47	34±3	0.81±0.12	35±3	0.83±0.17
49	42±3	2.9±0.21	41.3±2	2.8±0.47
51[3]	26±4	3.0±0.2	-	-
Gramicidin[3]	43±4	5.0±0.4	-	-
Valinomycin[3]	51±3	34±0.7	-	-

1)Maximum rate of transport; mol H^+ s^{-1} ($x10^{10}$)
2)Concentration of cation to obtain half the maximum rate; mM
3)From the Masters thesis of K. C. Kaye[60].

The selectivity of many of the compounds for the transport of alkali metal cations was determined. This proved to be the most important factor in this particular study for the evaluation of the modes of transport. Table 21 shows the rates observed.

Inhibition of either K^+ or Na^+ by added Li^+ also proved useful in the evaluation of the modes of transport. Table 22 shows the inhibition studies carried out.

Table 21. The metal ion selectivities of selected transporters as determined by the rate of ion transport.[4]

Table 21 Mimic	Li	Na	K	Rb	Cs
	Rate[1] $1x10^{-10}$	Rate[1] $1x10^{-10}$	Rate[1] $1x10^{-10}$	Rate[1] $1x10^{-10}$	Rate[1] $1x10^{-10}$
62[2]	4	41	27	28	31
63	3	27	18	25	25
44	1	20	40	35	28
45[2]	2	16	18	19	14
47	1	11	19	19	15
49	4	95	29	44	45
51	5	14	17	25	20
50	1	9	48	43	17
Valin[3]	~0	2	26	30	19

1)Rates calculated as a first order process, unless otherwise noted. Units: mol H^+ s^{-1}.
2)Rates calculated as a zero order process. Units: mol H^+ s^{-1}
3)Valinomycin
4)Table 23 (pages 107-116) contains details for each experiment

Table 22. Inhibition studies; the attempted inhibition of "fast" moving metal ions by "slower" moving metal ions.[3]

Table 22 Mimic	M+	[M+] mmol	Rate[1] $1x10^{-10}$
44	K	95	40
44	K:Li	93:17	41
45[2]	K	93	18
45[2]	K:Li	93:17	18
47	K	94	25
47	K:Li	93:17	28
49	Na	95	59
49	K:Na	93:17	27
49	Na	95	95
49	Na:Li	94:18	42
50	K	94	35
50	K:Li	93:17	31
51	K	96	17

Table 22 Mimic	M+	[M+] mmol	Rate[1] $1x10^{-10}$
51	K:Li	93:17	13
62[2]	Na	94	38
62[2]	Na:Li	93:17	28
63	Na	93	27
63	Na:Li	93:17	24

1)Rates calculated as a first order process, unless otherwise noted. Units: mol H^+ s^{-1}
2)Rates calculated as a zero order process. Units: mol H^+ s^{-1}
3)Table 23 (pages 107-116) contains details for each experiment.

DISCUSSION

What makes an active compound ?

The first important structural factor that has a consistent trend throughout the full range of the compounds is the head group. Both thioglucose and thioacetate head groups produce active compounds whereas in all cases of thiopropanol headgroups produce inactive compounds. The reasons for this variation in activity can be tentatively ascribed to the small size and insufficient polarity of the thiopropanol group. It is also possible that a small non-polar group may have unfavourable interactions with the head groups of the phospholipid membrane or may not partition from the membrane and into

the aqueous phase sufficiently to form an active compound.

The next factor involves the walls: molecules which possess wall units derived from **2** and **7** are the most active, compounds with a wall unit from **19** are less active and compounds which have wall units from either **24** or **10** are essentially inactive. These inactivities can be explained in terms of the length of the wall; compounds possessing a **24** wall are apparently too long and compounds having a **19** wall are too short. From the activities (Table 17) it can be seen that too short is not as serious a problem as too long. This may be associated with the observation that lipid membranes can constrict and become thinner. Membranes are known to constrict or "dimple" to facilitate the activity of gramicidin[51]. However in the case of the longer walls the membrane is not able to compensate and reorganisation of the transporter must occur. In our case this compensation by the transporter molecule results in an inactive system. Perhaps the molecule becomes too loosely packed or too tightly packed; either of these factors would result in the destruction of any ordered passage for the metal ion through the membrane.

Inactivity in the system derived from **10** is less clear. The molecule is the required length by design but from other work, particularly of Zojaji[37], it has been found that the molecule can adopt a "shorter" conformation. Zojaji isolated a capped molecule when he reacted this macrocycle with a bisthiol: he did not detect any similar capping with **7** or **2**. The result implies that the two alkenes can be in closer proximity in **10** than in the other two macrocycles. A

compressed or bent structure can be envisioned.

Interestingly the activity of the compounds is not di: .ctly affected by the central core. The compounds have a spectrum of activities with no apparent correlation with the number of arms appended to the crown ether.

What can be gleaned from analysis of concentration dependencies ?

Transporters that exhibit zero order kinetics exhibit a lower order in transporter than those that exhibit first order kinetics. This merely indicates that the transporter is not involved to the same degree in the rate process. If the rate process is channel gating, the zero order/first order distinction must then be linked to a difference in gating behaviour.

Compound **49** exhibits anomalous biphasic behaviour which can be explained by aggregation. At low concentrations the active system is that of a unimolecular species. Higher concentrations result in the formation of aggregated species that are also active transporters (pores). The observed increase in the apparent order above 0.6mM is consistent with this proposal.

The apparent kinetic order of the transporter gives little information on its mode of action. The results for gramicidin (a channel) and valinomycin (a carrier) are insignificantly different. However, as with the case of compound **49**, a change in apparent order is useful in assigning a change on the molecular level.

The apparent order in metal ions is of even less value in ascribing

mechanism. However for analysis of the metal ion selectivities it is reassuring to know that the concentrations used are far into the saturation range.

What does the selectivity tell us ?

In this transport system, gramicidin exhibits no selectivity for any of the alkali metals[50], whereas valinomycin shows high selectivity for potassium and rubidium relative to the other metal ions. This particular selectivity with a maximum at potassium or rubidium is characteristic of processes that involve the equilibrium exchange of an alkali metal from water into an environment of oxygen donors, Eisenman cation selectivity sequences III or IV[52].

The selectivity of compound **50** follows this same pattern but compound **49** shows a marked selectivity for Na^+ relative to all other cations. The selectivity order for compound **49** fits no Eisenman selectivity sequence indicating that the rate process is not governed by equilibrium effects. These two compounds have the same linkages and groups but differ only in the configuration of the crown core. The compound with the *meso* linkage can only possess an *anti* disposition of wall units at the expense of the crown cavity[40]. The selectivity of this compound compared with the valinomycin selectivity suggests that this compound behaves like valinomycin (a carrier). Compound **49** has a selectivity that is unlike valinomycin but is also unlike gramicidin.

The selectivities of a spectrum of active compounds were investigated. The selectivities can be broken down into two classes, Eisenman and non-

Eisenman, with many of the non-Eisenman compounds showing a spike in selectivity for sodium. Compound **51** shows Eisenman selectivity II; this is the same selectivity exhibited by gramicidin single channel conductances in black lipid membranes and for ion diffusion in dilute aqueous solution[51]. Out of the eight compounds whose selectivities were studied, three possess Eisenman selectivity III or IV; compounds **44** and **50** have Eisenman selectivity IV and compound **47** is either III or IV (the rate of transport for rubidium and potassium are the same). Compound **51**, as mentioned earlier, has selectivity II, and the remaining four compounds studied have no Eisenman selectivity: compounds **62**, **63**, **45** and **49**.

Carrier or Channel ?

A more useful tool in the differentiation of mechanism (channel versus carrier) is a study of inhibition. The inhibition of a carrier requires a special set of circumstances based on the balance of the forward and return rates. For a diffusion limited case this is equivalent to a balance of the extraction and release equilibrium extraction constants for substrate and inhibitor. Inhibition of a channel is easily explained by simple analogy: when a pipe is blocked by a slow moving object the passage of a faster moving species is hindered. This version of inhibition implies that metal ion movement through the membrane must be through the crown core. Inhibition of the sodium rate by lithium ion was observed for all compounds that possess the spike feature in the metal ion

selectivities. For compound **49** the sodium rate was also inhibited by the addition of potassium. The reproducibility is ±10% so only effects that lie substantially outside this range are taken as significant. Compounds **62, 51,** and **49** have significant inhibition and compound **63** is a borderline case. The compounds that possess Eisenman selectivities similar to valinomycin display no inhibition of K^+ transport by Li^+ and in some cases the rate is accelerated. Acceleration in the rate can readily be explained by a carrier mechanism since the overall rate will increase with the number of ions to be transported.

Interestingly, all the active compounds prepared that have two wall units plus the *meso* four-walled compound have Eisenman III and IV selectivities and are not inhibited by lithium. This leads to the general conclusion that these particular compounds act with the carrier mechanism. All other active compounds exhibit non-Eisenman selectivities or selectivity II like gramicidin in the case of compound **51**, and most are inhibited by lithium. Kaye carried out inhibition studies on compound **51** using octyl ammonium sulphate. Together with the activation energy determined for the compound (16kJ/mol), Kaye argues that compound **51** is an ion channel. Such low activation energies are characteristic of a channel mechanism[21]. The remaining active compounds have significant similarities to both gramicidin and compound **51** and insignificant similarities with valinomycin. Consequently the remaining compounds are apparently ion channels.

What information does "add back" and the plateau height give ?

The plateau behaviour is exhibited by gramicidin and amphotericin in the Herve *et al.*[45] vesicle system and by compound **51** in this vesicle system. Gramicidin and amphotericin stay essentially within the first lamella entered. This implies that once that vesicle contents have been emptied, the transporter is no longer effective. Any vesicles which do not contain active transporters remain intact throughout the experiment. The plateau height then shows the fraction of vesicles containing an active transporter. This argument is correct for the transport analysis of gramicidin, amphotericin and compound **51** since for all these compounds the extent and rate are proportional to the transporter concentration.

Valinomycin does not display this plateau behaviour. Valinomycin readily moves between lamellae so all the vesicles within a given system are emptied irrespective of the transporter concentration. Compounds **44** and **50** from their selectivities and lack of inhibition are believed to act like the carrier valinomycin. Of these compounds, compound **50** is the most obviously similar to valinomycin in its behaviour. At all concentrations the rate is proportional to, and the extent of transport is independent, of concentration. With compound **44** at very low concentrations the extent of transport is proportional to concentration; for higher concentrations it is independent. This result shows that although the analysis used by Herve *et al.*[45] may help confirm a particular mechanism it is not a criterion from which mechanism can be directly inferred.

It is just a lucky coincidence that the rate for movement between vesicles in these systems lies on either side of the transport rate; for valinomycin this rate is faster and for gramicidin it is slower. With compound **44** the balance between the transport and transfer rates must be close and changing the concentration tips this balance.

The most complicated example and yet the most interesting case is that of compound **49**. Here, biphasic behaviour is exhibited by this compound in the rate as a function of concentration. (**Figure 51**) The analysis of this biphasic behaviour using the method of initial rates shows that aggregation may be responsible for the second region. This postulate is confirmed by the plateau analysis for the first region where the rate is proportional to the extent of transport and for the second region where the extent of transport is independent of the rate of transport. If aggregation is the cause of this differential rate then the transporter added must enter the same vesicle in multiple copies. Since the extent is at the maximum, the transporter has apparently satiated all vesicles.

From add back experiments all the transporters studied do move between lipid layers, but those with six arms are less mobile than those with four arms. Compound **51** has a rate after add back that is 25% of that observed initially. The results obtained for the add back experiments agree well with the extent of transport arguments; compounds that display concentration dependent plateau behaviour display the expected low add back

activity. Compounds which show the maximum extent of transport at all concentrations show high activity after add back (with concentration effects taken into account). Compounds that are promiscuous display high add back rates and high extents of transport independent of concentration; compounds that slowly move between vesicles with low add back rates show low extents of transport which are dependent on concentration. The conclusions made by Herve *et al.*[45], that extent of transport should be proportional to the rate of transport for a channel and independent of rate for a carrier, have from this work been shown to be restrictive and limiting cases.

A more detailed analysis ?

For a clearer discussion of the experimental system a simple model can be proposed. The model focuses on three important process events: transporter movement between vesicles (P_1); gating or activation of the transporter (P_2); and, ion transport (P_3). For a carrier P_2 and P_3 are identical; gating and activation is ion transport for a carrier. (**Figure 55**)

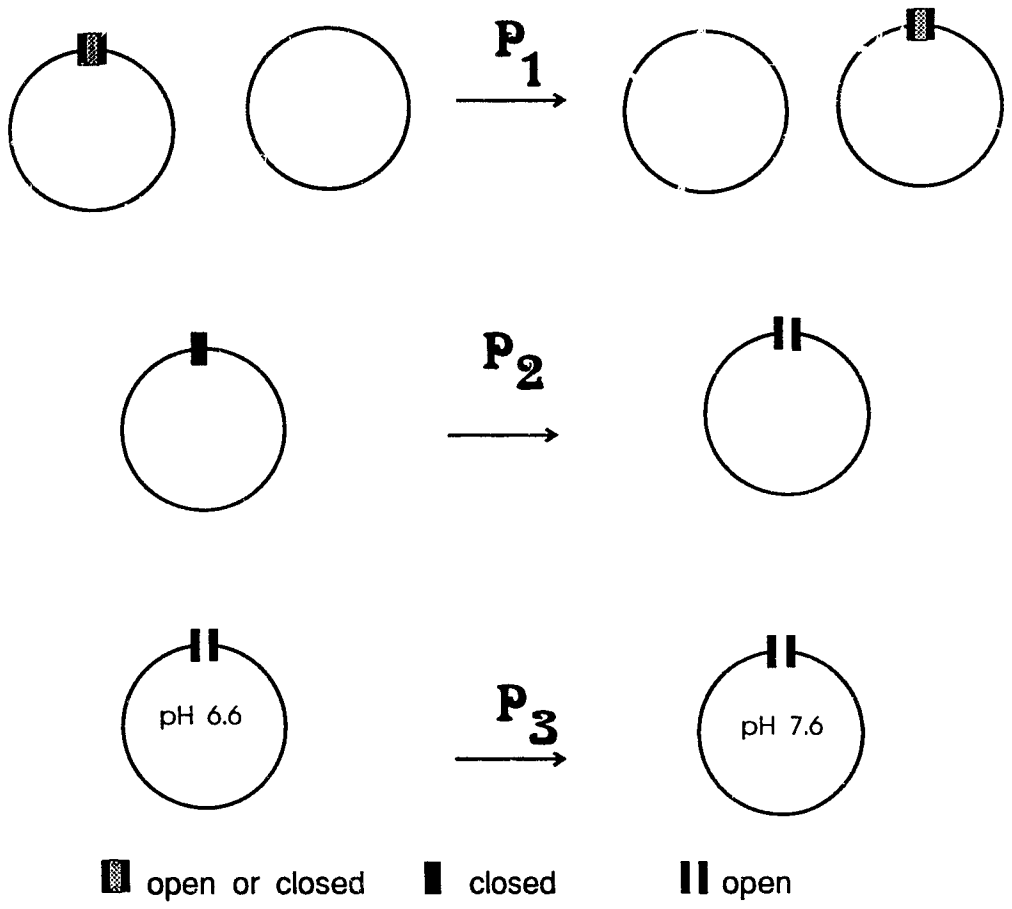


Figure 55. Schematic of the important processes involved in channel mediated ion transport.

The gramicidin behaviour is easy to explain using this cartoon analysis. Gramicidin is fixed within a membrane once it has entered; therefore, P_1 can be neglected. As mentioned earlier P_3 is very rapid for gramicidin, making the activation or gating process, P_2, rate determining. For valinomycin P_1 can also be neglected since movement between vesicles is rapid; the ion movement step is important $P_{(2,3)}$. Both gramicidin and valinomycin display a first order decay of the imposed pH gradient across the vesicle membrane in a pH-stat

analysis. For gramicidin this is explained, as mentioned earlier, by the random nature of the gating process P_2. With valinomycin first order decay is observed because the imposed gradient decays exponentially.

Within the set of compounds I investigated, compounds that mimicked gramicidin (compound **51**) and valinomycin (compounds **55**, **44**, **47**, **50**, **48**) were discovered, and other compounds behaved anomalously or were inactive. For example, compound **49**, from this study is believed to act via a channel mechanism but it is promiscuous, so displays pH-stat curves which at high transporter concentrations resemble the behaviour of valinomycin more than gramicidin. The major anomalies discovered were the zero order systems, compounds **45**, **62** and **46**, which are also believed to act via a channel mechanism on the basis of selectivity and inhibition results. For these systems, P_1 is not the important factor since the results from add back experiments show these compounds to be promiscuous. The gating process, P_2, is then the rate governing process for zero order channels, as it is with first order channels more like gramicidin.

How could channel mediated transport be zero order ?

One way to picture a zero order process requires that the channel opening event be of "intermediate" duration; "slow" with respect to the movement of a single metal ion but "fast" with respect to equilibration of a vesicle. The first order channels are "fast": once they open, the vesicle is

equilibrated during that single event. The observed rate is then directly related to the number of intact vesicles remaining (exponential decay). With an "intermediate" opening duration the rate process observed does not correspond to the number of remaining intact vesicles. A vesicle can continue to release protons for the pH-stat experiment over a long period of time involving several channel openings. Here a balloon analogy can be used. A first order channel "pops" a population of balloons randomly until no more remain (rate is proportional to the number of remaining balloons). A zero order channel "bleeds" the balloon by letting the air out in short bursts. The same number of balloons exist at all times, each balloon containing less air than initially (rate is independent of the population within a set of balloons).

How does gating occur ?

The active form of a gramicidin mimic requires a clear tube for the ions to pass through. Whether such a clear channel is formed in the mimics is not known. Any path through the mimic could be blocked by an alkyl chain of a phosphatidyl choline mixing with the mimic. The strength of the interactions of the alkyl chain of the phosphatidyl choline with the channels walls could determine the lifetime of the inactive species. The activity of the mimic may also be modulated by conformational fluctuations as with the gramicidin mimic of Stankovic, Heinemann and Schneiber[12]. Here some conformational change results in a switching between an open and closed state. In the case of our ion

channels this could be the result of a minimal orientation of wall units in parallel.

Only compounds with the wall unit derived from **2** exhibit zero kinetic order. As only one structural type exhibits this order, some conformational gating could be implicated. This type of wall will have different conformations from that exhibited by active compounds possessing other walls. The insertion of the alkyl chain of phosphatidyl choline into a channel formed by these compounds is very likely. This molecule has no polar functionality along the wall units so hydrophobic interactions with the alkyl chain of the phosphatidyl choline will be strong.

What more is needed ?

Obviously the story is not complete and other measurements are required before a channel mechanism can be ascribed unequivocally to these compounds. In particular the most unambiguous method for determining the mechanism is single channel unit conductance measurements. Initial studies on the Carmichael compound by Sansom[53] (**Figure 56**) showed the expected unit conductance steps associated with the channel mechanism of ion movement. This collaboration will hopefully continue and the compounds studied herein will also be investigated.

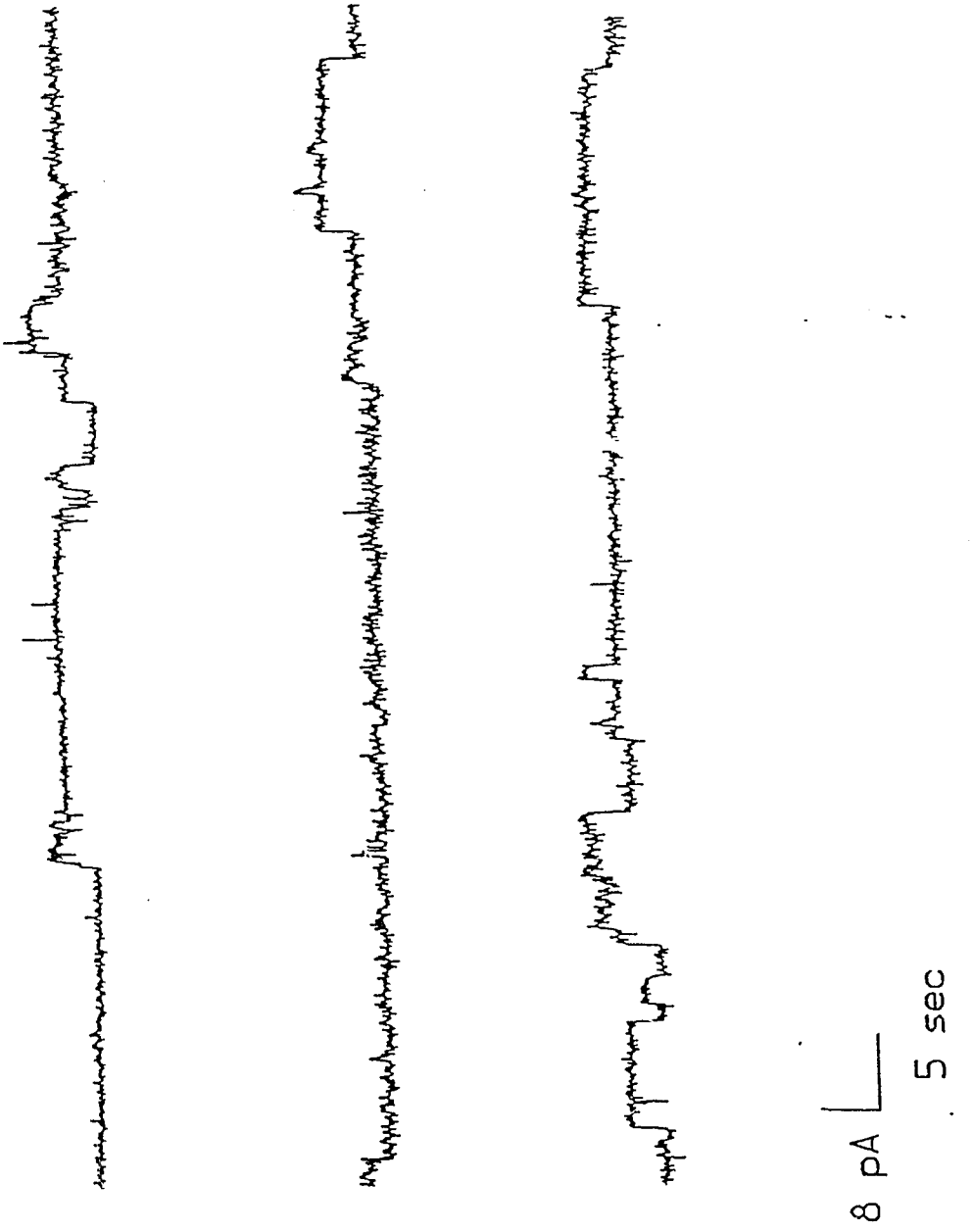


Figure 56. Conductivity *versus* time for the compound synthesised by Carmichael. Single channel conduction experiment performed by Sansom[53].

A conclusion ?

From this work it can be seen that creating an active compound by rational design is quite simple. In the survey conducted, active compounds that behave like the carrier valinomycin, or the channel gramicidin were discovered. The aim of this work was to predict the mode of action of these rationally designed molecules. This goal has been met to a limited degree. The head group variations required to make active compounds are still poorly defined. However, to obtain an inactive compound, regardless of mechanism, a small non-polar head-group should be used (thiopropanol). Wall length variations have shown that it is preferable to be too short rather than too long. Predictability of mechanism is a much tougher nut to crack. The limited scope of physical measurements carried out in this work indicate some minimum requirements for a mode of transport similar to gramicidin to be observed. The number of walls seems to be critical; the compounds with two walls behave like valinomycin, but compounds with four or six walls behave more like gramicidin.

A satisfying result, yet one which seems self serving, is the observation of the isomeric compounds **49** and **50**. Here a minor structural variation associated with the disposition of the carboxylates of the tartaric acid unit of this crown produces a compound with a drastically different mode of action. Compound **49** has a structure that correlates directly with the design structure; compound **50** however, does not correlate with the design structure.

The former is an ion channel; the latter is an ion carrier. (**Figure 57**)

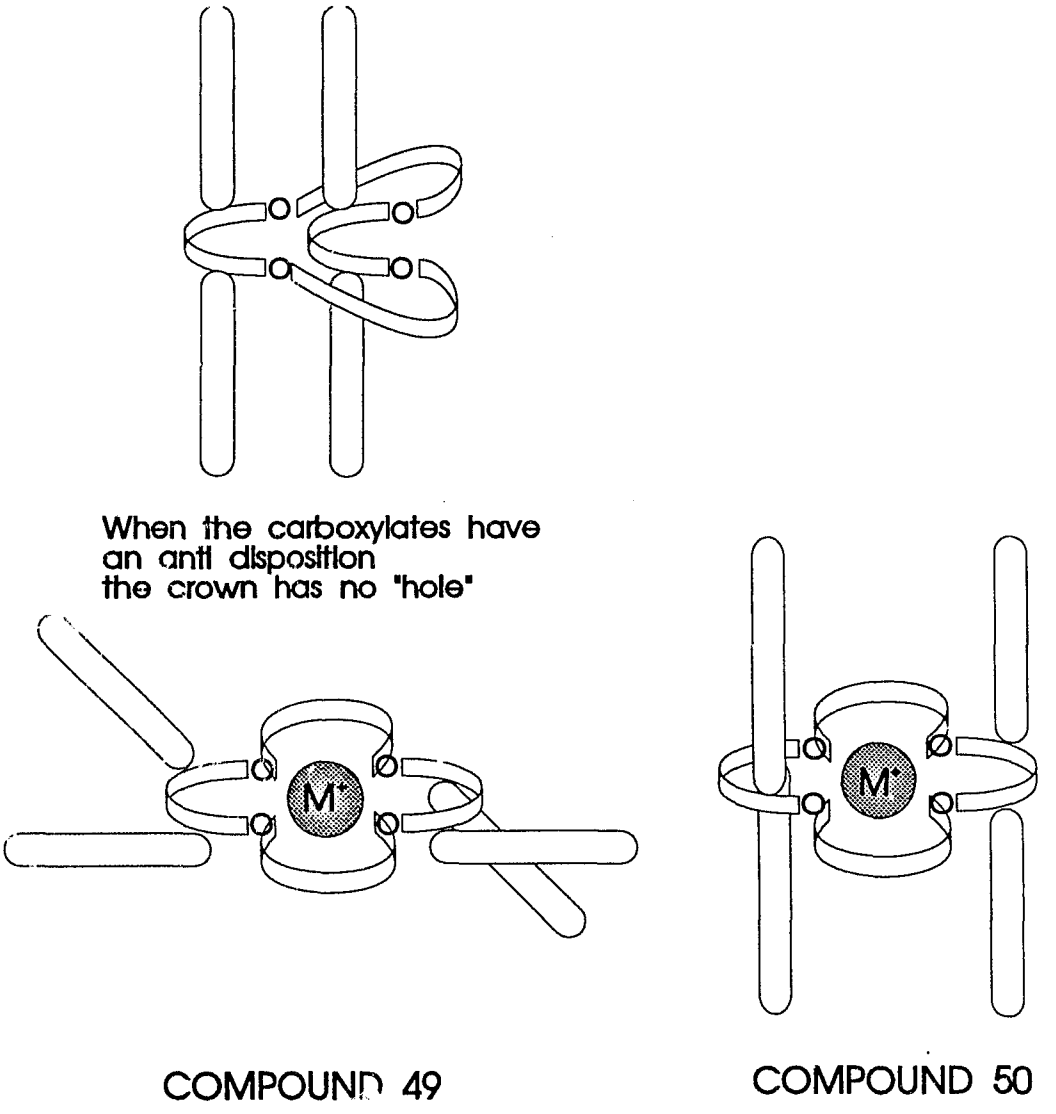


Figure 57. Cartoon structures for compounds **49** and **50**

In conclusion, the chicken and egg analogy must be used. The results presented agree almost exactly with the design criteria set out initially; so which came first ? It is gratifying to know that the design criteria preceded

the experimental results by several years[38,46].

A summary ?

A graphic summary of the results and conclusions reached from this study is given on the next page. (**Figure 58**) Repeating this summary in words: of the twenty-one compounds prepared nine were inactive (compounds **57**, **58**, **52**, **53**, **54**, **59**, **60**, **61** and **64**), five were carriers (compounds **44**, **47**, **48**, **55** and **50**), and seven were believed to be ion channels (compounds **51**, **49**, **46**, **45**, **56**, **63** and **62**). The ion channels discovered consist of two types, ascribed from the observed order of ion transport: three are zero order (compounds **46**, **45** and **62**) and four are first order (compounds **51**, **49**, **63** and **56**).

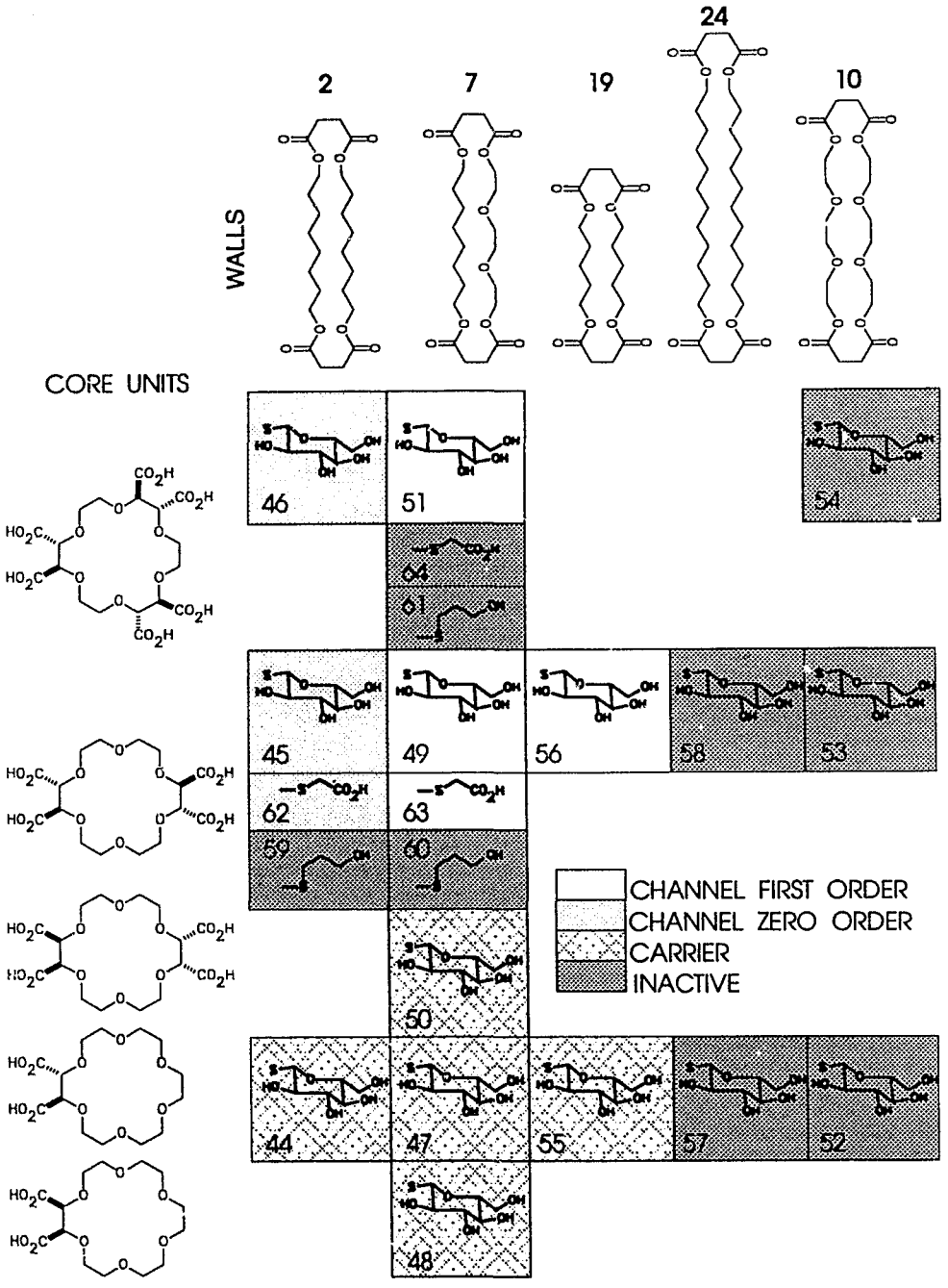


Figure 58. Schematic of the behaviour of all the channel mimic compounds synthesised. Refer to text for details.

Table 23. Results from the transport studies carried out on the twenty one compounds synthesised

Table 23 Mimic[8] (T)	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
			mM	μM	$1x10^{-10}$	%
44	7	Li	95.	1.0	1.0	~0
44	7	Na	95.	1.0	20.	77
44	7	Rb	95.	1.0	35.	90
44	7	Cs	95.	1.0	28.	83
44	7	K	95.	0.26	17.	60
44	7	K	95.	1.0	37.	90
44	7	K	95.	0.52	23.	82
44	7	K	95.	1.5	49.	88
44	7	K	95.	1.0	40.	93
44	9	K	95.	1.0	40.	89
44	5	K	95.	2.6	97.	95
44	7	K	96.	2.6	76.	92
44[4]	9	**K:Li**[4]	93:17[4]	1.0	41.	77

Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] **(T)**			**mM**	**μM**	$1x10^{-10}$	%
45	8	Li	95.	1.6	2.4[3]	**95**[2]
45	8	Na	94.	1.6	16.[3]	**95**[2]
45	8	Rb	94.	1.6	19.[3]	**95**[2]
45	8	Cs	94.	1.6	14.[3]	**95**[2]
45	10	K	93.	3.2	18.[3]	**95**[2]
45	8	K	94.	1.6	18.[3]	**95**[2]
45	4	K	94.	0.32	8.2[3]	**95**[2]
45	4	K	95.	2.3	19.[3]	**95**[2]
45	4	K	95.	0.65	12.[3]	**95**[2]
45	4	K	95.	1.6	16.[3]	**95**[2]
45	4	K	95.	3.2	23.[3]	**95**[2]
45[4]	10	**K:Li**[4]	93:17[4]	3.1	18.[3]	**95**[2]
46	4	K	94.	2.0	8.0[3]	**95**[2]
47	6	Li	94.	1.8	1.0	~0
47	6	Na	93.	1.8	11.	61

Table 23 Mimic[8] (T)	Batch[5]	M^+	$[M^+]$ mM	[T] µM	Rate[1] $1x10^{-10}$	Extent[7] %
47	6	Rb	92.	1.8	19.	94
47	6	Cs	93.	1.8	15.	69
47	6	K	93.	1.8	19.	83
47	7	K	93.	3.0	36.	77
47	9	K	94.	3.0	25.	92
47	5	K	95.	3.1	32.	94
47	7	K	20.	3.3	32.	75
47	7	K	10.	3.3	33.	71
47	7	K	8.2	3.3	29.	69
47	7	K	6.1	3.3	34.	54
47	7	K	2.0	3.3	22.	44
47	7	K	1.0	3.3	20.	36
47[4]	9	**K:Li**[4]	93:17[4]	3.0	28.	72
48	1	K	98.	2.0	7.3	64
48	5	K	95.	2.8	27.	80

Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] (T)			mM	µM	$1x10^{-10}$	%
49	5	Li	95.	0.51	4.1	20
49	5	Na	95.	0.51	95.	93
49	9	Na	95.	0.63	59.	98
49	5	Rb	94.	0.51	44.	98
49	5	Cs	94.	0.50	45.	103
49	3	K	99.	0.66	27.	84
49	7	K	93.	0.62	31.	93
49	3	K	98.	0.79	44.	81
49	3	K	99.	0.13	9.1	38
49	3	K	99.	1.3	100.	81
49	5	K	95.	0.51	29.	88
49	3	K	98.	0.66	28.	80
49	3	K	99.	0.26	14.	48
49	3	K	99.	0.53	25.	91
49	1	K	98.	0.66	21.	84

Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] **(T)**			mM	μM	$1x10^{-10}$	%
49	4	K	95.	0.63	25.	79
49	3	K	98.	0.92	63.	83
49	3	K	99.	0.40	16.	81
49	7	K	20.	0.68	34.	80
49	7	K	10.	0.68	35.	77
49	7	K	8.1	0.68	31.	64
49	7	K	6.1	0.68	28.	68
49	7	K	4.1	0.69	24.	55
49	7	K	2.1	0.69	17.	59
49	7	K	1.0	0.68	20.	44
49[4]	9	**K:Na**[4]	93:18[4]	0.62	27.	88
49[4]	5	**Na:Li**[4]	94:18[4]	0.50	42.	64
50	6	Li	93.	0.57	1.0	~0
50	6	Na	92.	0.57	8.9	53
50	6	Rb	93.	0.57	43.	98

Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] (T)			mM	µM	$1x10^{-10}$	%
50	6	Cs	92.	0.57	17.	87
50	2	K	98.	1.2	44.	81
50	1	K	98.	0.60	15.	72
50	3	K	97.	0.72	33.	77
50	3	K	98.	1.2	45.	98
50	1	K	98.	1.2	23.	[illegible]
50	3	K	99.	0.36	19.	72
50	9	K	94.	0.58	35.	97
50	6	K	93.	0.57	48.	94
50	1	K	98.	0.12	6.1	60
50	3	K	99.	0.61	24.	84
50	3	K	99.	0.12	8.0	98
50[4]	9	**K:Li**[4]	93:18[4]	0.57	31.	92
51	9	Li	95.	2.2	5.1	22
51	9	Na	96.	2.2	14.	61

Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] **(T)**			mM	µM	$1x10^{-10}$	%
51	9	Rb	95.	2.2	25.	78
51	9	Cs	96.	2.2	20.	88
51	1	K	98.	1.6	9.7	46
51	1	K	98.	0.78	4.6	37
51	1	K	98.	3.9	22.	66
51	2	K	98.	0.76	6.3	39
51	6	K	92.	1.4	10.	60
51	2	K	100.	0.79	5.7	49
51	9	K	96.	2.2	17.	70
51[4]	9	K:Li[4]	93:18[4]	2.2	13.	59
52	7	K	92.	2.2	4.6	92
53	4	K	95.	2.2	1.0	~0
54	4	K	95.	3.5	5.0	49
55	5	K	95.	3.3	22.	71
56	4	K	95.	1.3	7.8	60

Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] **(T)**			mM	µM	$1x10^{-10}$	%
56	4	K	94.	6.3	29.	97
56	2	K	100.	1.3	7.5	69
57	5	K	92.	3.0	1.0	~0
58	4	K	95.	3.1	3.2	~0
59	4	K	95.	2.4	1.0	~0
60	2	K	98.	1.4	2.6	97
60	2	K	98.	2.3	6.4	95
61	2	K	98.	1.6	1.1	17
61	2	K	98.	3.1	2.8	7
62	8	Li	92.	2.0	3.8[3]	**95**[2]
62	8	Na	94.	2.1	38.[3]	**95**[2]
62	8	Na	93.	2.0	41.[3]	**95**[2]
62	8	Rb	94.	2.1	28.[3]	**95**[2]
62	8	Cs	93.	2.0	31.[3]	**95**[2]
62	4	K	95.	4.1	18.[3]	**95**[2]

Table 23 Mimic[8] (T)	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
			mM	μM	$1x10^{-10}$	%
62	8	K	93.	2.0	27.[3]	**95**[2]
62	4	K	95.	1.2	14.[3]	**95**[2]
62	4	K	95.	2.1	17.[3]	**95**[2]
62[4]	8	**Na:Li**[4]	93:17[4]	2.0	28.[3]	**95**[2]
63	10	Li	92.	2.4	3.4	29
63	10	Na	93.	2.4	27.	78
63	10	Rb	93.	2.4	25.	76
63	10	Cs	93.	2.4	25.	78
63	10	K	93.	2.4	18.	74
63	2	K	98.	2.6	21.	43
63	2	K	98.	0.51	6.0	34
63	2	K	98.	1.3	11.	45
63[4]	10	**Na:Li**[4]	93:17[4]	2.4	24.	80
64	10	Li	94.	3.2	1.9[3]	**95**[2]
64	10	Rb	93.	3.2	5.8[3]	**95**[2]

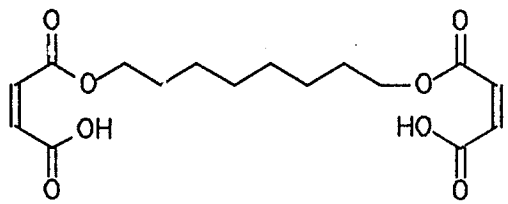
Table 23	Batch[5]	M^+	$[M^+]$	[T]	Rate[1]	Extent[7]
Mimic[8] **(T)**			mM	μM	$1x10^{-10}$	%
64	10	Na	93.	3.2	5.2[3]	**95**[2]
64	10	Cs	93.	3.2	6.6[3]	**95**[2]
64	10	K	93.	3.2	4.1[3]	**95**[2]
64[4]	10	**Na:Li**[4]	93:17[4]	3.1	5.3[3]	**95**[2]
Gram.[6]	5	K	94.	0.025	66.	56

1)Rates calculated using a first order analysis, unless otherwise noted. Units: mol H^+ s^{-1}
2)Multilameller limit determined by melittin assay
3)Rates calculated using a zero order analysis. Units: mol H^+ s^{-1}
4)Inhibition studies: M^+_1/M^+_2 ratio is 5/1
5)Indicates batch of vesicles that the measurements were carried out in.
6)Gramicidin
7)Extent of transport as a percentage of the lyse volume; calculated from the observed or calculated plateau height and the observed lyse volume.
8)FCCP concentration was 0.15-0.18μM.

EXPERIMENTAL

Melting points were taken on a Reichart hotstage microscope (uncorrected). Proton nmr spectra were recorded with a Perkin Elmer R32 (90MHz, CW) or Bruker AMX 360 (360.14MHz, FT) spectrometers in $CDCl_3$, CD_2Cl_2, or CD_3OD as solvent, 90MHz ^{1}HNMR spectra (R32) were referenced to Me_4Si as internal standard, and all 360MHz ^{1}HNMR spectra (AMX 360) were referenced with the central solvent line as standard (7.24δ for $CDCl_3$, 5.32δ for CD_2Cl_2 and 3.30δ for CD_3OD all relative to Me_4Si). Carbon spectra were recorded with either a Bruker WM 250 (62.89MHz) or Bruker AMX 360 (90.57MHz) with the central solvent line as standard (77.0ppm for $CDCl_3$, 53.8ppm for CD_2Cl_2 and 49.0ppm for CD_3OD all relative to Me_3Si). Mass spectra were recorded on a Finnegan 3300 GC-MS instrument with methane chemical ionisation. Elemental analyses were performed by Canadian Microanalytical Services, New Westminster, B.C., and are quoted as percentages.

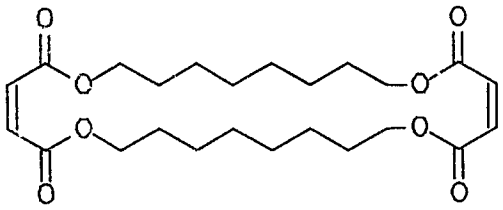
5,14-dioxa-4,15-dioxooctadeca-2,16-diene-1,18-dicarboxylic acid (1)



The 1,8-octanediol (146g, 1mol) and maleic anhydride (196g, 2mol, 2eq)

were mixed in benzene (500mL) and refluxed for 6h. The benzene was removed under reduced pressure to give **1** as a colourless solid (342g, 1mol, quantitative): ^{1}HNMR 90MHz (δ, $CDCl_3$) 9.8 (br s, 2H, 2xCO_2***H***), 6.2 (s, 4H, 2x*cis*C***H***=C***H***), 4.1 (t, J=6Hz, 4H, 2xC***H***$_2$O), 1.5 (m, 2xC***H***$_2$ β), 1.2 (br. s, 4xC***H***$_2$ δ, γ); ^{13}CNMR 62.89MHz (δ, $CDCl_3$) 167.1 (***C***=O), 166.5 (***C***=O), 133.4 (CH=***C***H), 130.5 (*cis****C***H=CH), 66.5 (2x***C***H$_2$O), 28.8 (2x***C***H$_2$ β), 28.1 (2x***C***H$_2$ γ), 25.5 (2x***C***H$_2$ δ); MS (CI, m/e): 343(M+1)

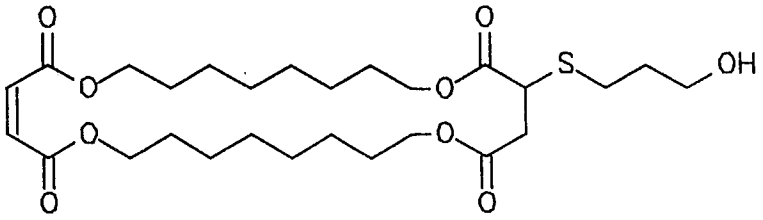
1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-3,17-diene (2)



Compound **1** (20.52g, 60mmol) and 1,8-octanediol (8.76g, 60mmol, 1eq) were mixed in benzene (1500mL). Methanesulphonic acid (15 drops) was added and the mixture was heated with azeotropic removal of water (Dean-Stark) for 12h. The solvent was removed under reduced pressure. The solid was triturated with diethylether (200mL), then recrystallised from ethyl acetate, to give **2** as a colourless crystalline solid (3.3g, 7.3mmol, 12%): mp 103-105°C; ^{1}HNMR 90MHz (δ, $CDCl_3$) 6.2 (s, 4H, 2x*cis*C***H***=C***H***), 4.2 (t, J=6.2Hz, 8H, C***H***$_2$O), 1.6 (m, 4xC***H***$_2$ β), 1.4 (br. s, 8xC***H***$_2$ δ, γ); ^{13}CNMR 100.12MHz (δ, $CDCl_3$) 165.2 (4x***C***=O), 129.6 (2x*cis****C***H=***C***H), 65.3 (4x***C***H$_2$O), 29.1 (4x***C***H$_2$ β),

28.4 (4xCH_2 γ), 25.8 (4xCH_2 δ); MS (CI, m/e): 453(M+1), 481(M+29), 493(M+41); Analysis calculated for $C_{24}H_{36}O_8$: C 63.7%; H 7.95%; Found C 63.6%; H 7.96%

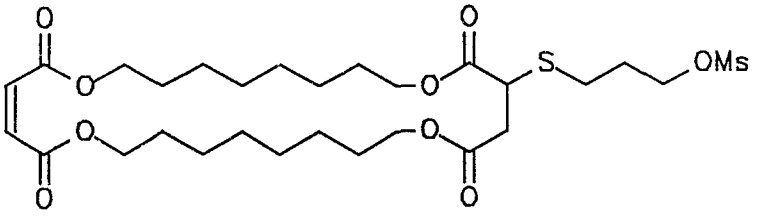
3-(4-hydroxy-1-thiabutyl)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (3)



Compound **2** (12.52g, 27.0mmol) and 3-mercapto-1-propanol (2.55g, 27.0mmol, 1eq) were added to 2-propanol (200mL). Piperidine (10 drops) was added and the mixture was heated at reflux for 1h 45min. The solvent was removed under reduced pressure. The product was then chromatographed on 8% deactivated alumina (250g) with a dichloromethane/hexane gradient (50:50 to 100% CH_2Cl_2). Unreacted **2** was obtained in the early fractions eluted with 50% dichloromethane; later fractions (50% to 100%CH_2Cl_2) contained the product. Washing the column with a 1% methanol in dichloromethane mixture yielded **4**. The combined product containing fractions were evaporated to give **3** as a clear oil (4.15g, 76mmol, 28%): ^{1}HNMR 90MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, C***H***$_2$O), 3.7 (m, 3H, C***H***$_2$OH, SC***H***), 3.2-2.7 (m, 4H, C***H***$_2$CHS, SC***H***$_2$), 2.4 (s, 1H, O***H***), 2.0-1.2 (m, 26H, C***H***$_2$); ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 24); MS (CI, m/e): 545(M+1); Analysis calculated for $C_{27}H_{44}O_9S$: C 59.54%; H 8.14%; S 5.89%; O 26.43%; Found C 60.04%; H 7.91%;

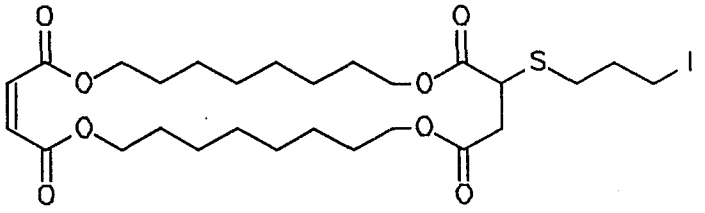
S 5.52%; O 25.95%.

3-(4-methanesulphonyl-1-thiabutyl)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (5)



Compound **3** (4.15g, 7.6mmol) and triethyl amine (4.04g, 40mmol, 5eq) were dissolved in dichloromethane (150mL) and the mixture cooled to -10°C. Methane sulphonyl chloride (1.72g, 15mmol, 2eq) in dichloromethane (5mL) was added dropwise over 30 minutes. The reaction was allowed to warm to room temperature and was stirred for a further 2h. The reaction mixture was then washed with saturated sodium chloride (2x100mL), 10% hydrochloric acid (2x100mL), 10% sodium bicarbonate (2x100mL) and again with saturated sodium chloride (2x100mL), dried over sodium sulphate, and the solvent removed to give **5** as a yellow oil (3.62g, 5.8mmol, 76%): ^{1}HNMR 90MHz (δ, $CDCl_3$), 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 10H, C$\boldsymbol{H}_2$O, C$\boldsymbol{H}_2$OMs), 3.6 (d of d, J=3Hz and 6Hz, 1H, C***H***S), 2.9 (s, 3H, $SO_2C\boldsymbol{H}_3$), 3.1-2.6 (br.m, 4H, C$\boldsymbol{H}_2$CHS, SC$\boldsymbol{H}_2$), 2.0 (m, 2H, C$\boldsymbol{H}_2CH_2S$), 1.8-1.2 (br m, 24H, C$\boldsymbol{H}_2$) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 24); MS (CI, m/e): 623(M+1)

3-(4-iodo-1-thiabutyl)-1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (6)

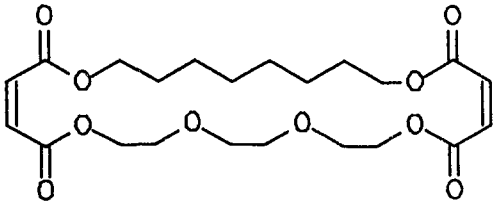


Compound **5** (3.62g, 5.8mmol) and sodium iodide (7g, 47mmol, 8eq) were mixed in acetone (100mL). The mixture was heated at reflux overnight. The solvent was removed under reduced pressure. The reaction mixture was dissolved in dichloromethane (200mL), washed with water (2x100mL), dried over sodium sulphate, and the solvent removed under reduced pressure, to give **6** as a yellow oil (3.0g, 46mmol, 79%): ^{1}HNMR 90MHz (δ, $CDCl_3$), 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, C***H***$_2$O), 3.65 (d of d, J=3Hz and 6Hz, C***H***S), 3.2 (t, J=6Hz, C***H***$_2$I), 3.0-2.7 (m, 4H, C***H***$_2$CHS, SC***H***$_2$), 2.1 (m, 2H, C***H***$_2$CH$_2$I), 1.8-1.3 (br m, 24H C***H***$_2$) ; ^{13}CNMR 62.89 ($CDCl_3$) (Refer to Table 24) ; MS (CI, m/e): 655(M+1); Analysis calculated for $C_{27}H_{43}O_8SI$: C 49.54%; H 6.62%; S 4.90%; I 19.39%; Found C 50.21%; H 6.56%; S 5.10%; I 19.64%

Table 24. ^{13}CNMR Data for compounds **3**, **5** and **6** (ppm)

Assignment	3	5	6
C=O	171.7	171.2	171.5
	170.6	170.3	170.4
	165.0	164.8	164.9
trans ***C*=*C***	133.5	133.4	133.5
CO_2CH_2	65.3	65.2	65.4
	64.9	64.8	64.9
CH_2CHS	41.7	41.5	41.5
CH_2CHS	36.6	36.4	36.6
CH_2	28.9	28.7	28.8
	28.4	28.2	28.4
	26.0	25.9	26.0
	25.3	25.2	25.3
CH_3SO_3		37.2	
CH_2X	61.1	67.8	4.3
CH_2S	31.8	28.6	32.0
CH_2CH_2X	28.1	27.3	32.4

1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-3,17-diene (7)

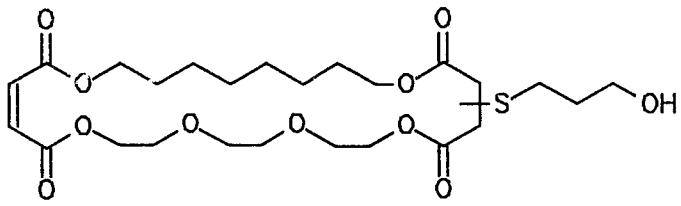


Compound **1** (20.52g, 60mmol) and triethleneglycol (9.0g, 60mmol, 1eq) were added to benzene (1000mL). Methanesulphonic acid (15 drops) was added and the mixture was heated with azeotropic removal of water (Dean-Stark) for 12h. The solvent was removed under reduced pressure and the yellow oil was combined with three other batches prepared analogously. The combined oils were then adsorbed from dichloromethane solution onto alumina (250g) and added to a column of silica gel (450g) prepared in 20% ethyl acetate/hexanes as a slurry. From this column four fractions were obtained; 20% ethyl acetate/hexanes (6L, **13** and **2**), 35% ethyl acetate/hexanes (4L, **14** and **7**), 50% ethyl acetate/hexanes (8L, **14** and **7**), 100% ethyl acetate (4L, **7** and **10**). The solvent was removed from fractions 2,3 and 4 and they were fractionated separately by Kugelrhor distillation, the first fraction collected as a clear oil (10^{-3}mmHg, 120°C) was **14**, the second fraction collected (10^{-3}mmHg, 220°C) was **7**, **10** remained as a yellow pot residue. The product containing fractions were combined to give **7** as a colourless solid (7g, 15.3mmol, 6.4%): ^{1}HNMR 90MHz (δ, $CDCl_3$): 6.2 (s, 4H, 2xCH=CH), 4.2 (m, 8H, 4xCO_2CH_2), 3.7 (m, 4H, 2x$CO_2CH_2CH_2O$), 3.6 (s, 4H, OCH_2CH_2O), 1.6 (m, 2xCH_2 β), 1.4 (br. s,

(m, 4H, 2x$CO_2CH_2CH_2O$), 3.6 (s, 4H, OCH_2CH_2O), 1.6 (m, 2xCH_2 β), 1.4 (br. s,

4xCH_2 δ, γ); ^{13}CNMR 90.57MHz (δ, $CDCl_3$) 165.1 (*C*=O), 165.0 (*C*=O), 130.2 (*C*H=CH), 129.0 (CH=*C*H), 70.5 (O*C*H_2*C*H_2O), 68.7 (2xCO_2CH2*C*H_2O), 65.2 (2xCO_2*C*H2CH_2O), 64.1 (2xCO_2*C*H_2), 28.7 (2x*C*H_2 β), 28.2 (2x*C*H_2 γ), 25.5 (2x*C*H_2 δ) ; MS (CI, m/e): 457(M+1); Analysis calculated for $C_{22}H_{32}O_{10}$: C 57.89%; H 7.07%; Found C 58.14%; H 7.10%

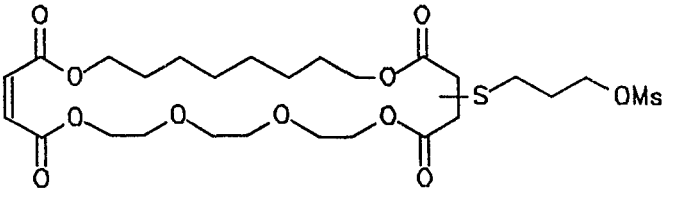
3-and4-(4-hydroxy-1-thiabutyl)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxo-cyclooctacosa-17-ene (8)



Compound **7** (5g, 11mmol) and 3-mercapto-1-propanol (1.0g, 11mmol, 1eq) were added to 2-propanol (150ml). Piperidine (10 drops) was added and the mixture was heated at reflux for 1h 30min. The solvent was removed under reduced pressure. The product was then chromatographed on 8% deactivated alumina (120g) with a dichloromethane/hexane gradient (50:50 to 100% CH_2Cl_2). Unreacted **7** was obtained in the early fractions eluted with 50% dichloromethane; later fractions (50% to 100% CH_2Cl_2) contained the product. Washing the column with a 1% methanol dichlorometane mixture yielded the product of double addition. The combined product containing fractions were evaporated to give **8** as a clear oil (2.38g, 4.3mmol, 39%): ^{1}HNMR 90MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C*H*=C*H*), 4.2 (m, 8H, CO_2C*H*$_2$), 3.6

(m, 11H, CH_2O, CH_2OH, SCH), 3.0-2.6 (m, 4H, CH_2CHS, SCH_2), 1.8-1.1 (m, 15H, CH_2, OH); ^{13}CNMR 62.89MHz ($CDCl_3$) (Refrer to Table 25) ; M. CI, m/e): 549(M+1)

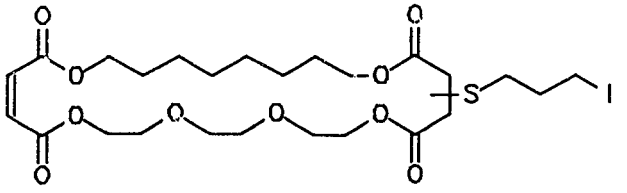
3-and 4-(4-methanesulphonyl-1-thiabutyl)-1,6,9,12,15,20-hexaoxa-2,5,16,-19-tetraoxocyclooctacosa-17-ene (9)



Compound **8** (720mg, 1.3mmol) and triethyl amine (2.02g, 20mmol, 15eq) were dissolved in dichloromethane (50mL) and the mixture cooled to -10°C. Methane sulphonyl chloride (860mg, 7.5mmol, 6eq) in dichloromethane (5mL) was added dropwise over 30 minutes. The reaction was allowed to warm to room temperature and was stirred for a further 2h. The reaction mixture was then washed with saturated sodium chloride (2x100mL), 10% hydrochloric acid (2x100mL), 10% sodium bicarbonate (2x100mL) and again with saturated sodium chloride (2x100mL), dried over sodium sulphate and the solvent removed to give **9** as a yellow oil (740mg, 1.2mmol, 90%): ^{1}HNMR 90MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* CH=CH), 4.2 (m, 10H, CO_2CH_2, CH_2OMs), 3.6 (m, 9H, CH_2O, SCH), 3.0 (s, 3H, SO_2CH_3), 2.9-2.6 (m, 4H, CH_2CHS, SCH_2), 2.0 (m, 2H, CH_2CH_2OMs), 1.8-1.3 (m, 12H CH_2) ; ^{13}CNMR 62.89MHz ($CDCl_3$)

(Refer to Table 25)

3-and 4-(4-iodo-1-thiabutyl)-1,6,9,12,15,20-hexaoxa-2,5,16,19-tetraoxocyc-looctacosa-17-ene (11)



Compound **9** (740mg, 1.2mmol) and sodium iodide (3g, 20mmol, 17eq) were mixed in acetone (100mL). The mixture was heated at reflux overnight. The solvent was removed under reduced pressure. The reaction mixture was dissolved in dichloromethane (200mL), washed with water (2x100mL), dried over sodium sulphate, and the solvent removed under reduced pressure, to give **11** as a yellow oil (500mg, 760µmol, 63%): ^{1}HNMR 90MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, $CO_2C\boldsymbol{H}_2$), 3.6 (m, 9H, $C\boldsymbol{H}_2O$, SC***H***), 3.2 (t, J=6Hz, $C\boldsymbol{H}_2I$), 2.9-2.6 (m, 4H, $C\boldsymbol{H}_2CHS$, $SC\boldsymbol{H}_2$), 2.0 (m, 2H, $C\boldsymbol{H}_2CH_2I$), 1.7-1.2 (m, 12H, $C\boldsymbol{H}_2$) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 25) ; MS (CI, m/e): 659(M+1)

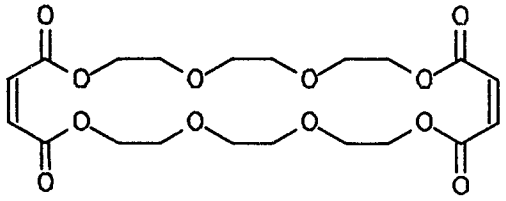
Table 25. ^{13}CNMR Data for compounds **8**, **9** and **11** (ppm)

Table 27 Assignments	**8**	**9**	**11**
C=O	171.6	171.3	171.3
	170.5	170.3	170.3
	164.9	164.9	164.8

Table 27 Assignments	**8**	**9**	**11**
trans *C*H=*C*H	134.0	134.0	134.0
	133.1	133.1	133.0
Ether	70.7	70.7	70.7
CH_2O	70.6	70.6	70.6
	70.4	70.4	70.4
	69.2	69.2	69.1
	69.0	69.0	69.0
	68.9	65.3	65.3
	65.3		
	65.2		
CO_2CH_2	64.7	64.8	64.8
	64.5	64.5	64.5
	64.4	64.3	64.4
	64.2	63.9	64.3
	63.9		63.9
CH_2CHS	41.7	41.6	41.5
	41.5	41.5	41.4
CH_2CHS	36.5	36.4	36.4
	36.4	36.3	36.3

Table 27 Assignments	8	9	11
CH_2	28.8	28.8	28.8
	28.6	28.5	28.6
	28.4	28.4	28.5
	28.3	28.3	28.3
	25.6	25.6	28.2
	25.3	25.3	25.6
			25.3
CH_3SO_3		37.3	
CH_2X	60.9	67.8	4.4
CH_2S	31.8	Hidden	32.0
	31.7		31.9
CH_2CH_2X	28.0	27.4	32.3

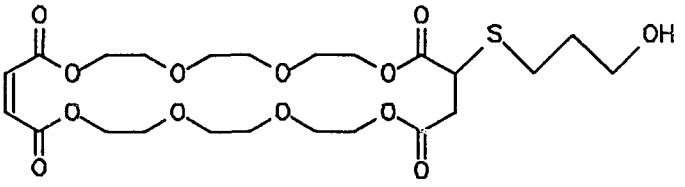
1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-3,17-diene (10)



The triethylene glycol (10g, 67mmol) and maleic anhydride (13.1g, 134mmol, 2eq) in benzene (1500mL) were refluxed for 3h. Triethlene glycol

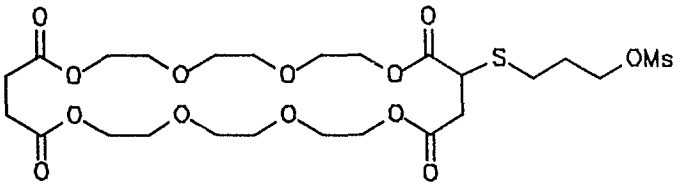
(10g, 67mmol) and methanesulphonic acid (15 drops) were added and the mixture was heated with azeotropic removal of water (Dean-Stark) for 12h. The solvent was removed under reduced pressure and the yellow oil combined with two batches made analogously. The oil was adsorbed from benzene onto alumina (200g) and added to a column of silica gel (250g) prepared in 50% ethyl acetate/hexanes as a slurry. Three fractions were collected; 50% ethyl acetate/hexanes (3L, **14**), 60% ethyl acetate/hexanes (1L, **14**), 100% ethyl acetate (3L, **10**). The solvent was removed from the third fraction and evacuated for 24h (the procedure can be improved subsequently by seeding before evacuation). The solid was then triturated with the minimum ethyl acetate to give **10** as a colourless solid (6.5g, 15.2mmol, 7.5%): ^{1}HNMR 90MHz (δ, $CDCl_3$) 6.2 (s, 4H, 2xC***H***=C***H***), 4.2 (t, J=3Hz, 8H, 4x$CO_2C\boldsymbol{H}_2$), 3.6 (m, 8H, 4x$CO_2CH_2C\boldsymbol{H}_2O$), 3.5 (s, 8H, 2x$OC\boldsymbol{H}_2C\boldsymbol{H}_2O$),; ^{13}CNMR 62.89MHz (δ, $CDCl_3$) 165.0 (4x**C**=O), 129.0 (2x**C**H=**C**H), 70.5 (2x$O\mathbf{C}H_2\mathbf{C}H_2O$), 68.8 (4x$CO_2CH_2\mathbf{C}H_2O$), 64.3 (4x$CO_2\mathbf{C}H_2CH_2O$); MS (CI, m/e): 461(M+1), 489(M+29), 501(M+41) ; Analysis calculated for $C_{20}H_{28}O_{12}$: C 52.17% H 6.13% Found C 51.9% H 6.03%

3-(4-hydroxy-1-thiabutyl)-1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-3,17-ene (12)



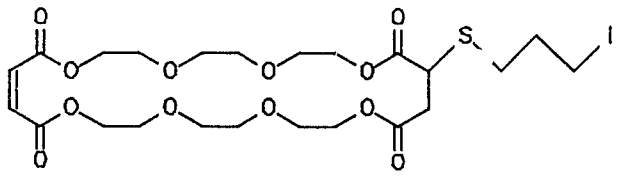
Compound **10** (6.4g, 14mmol) and 3-mercapto-1-propanol (1.3g, 14mmol, 1eq) were added to tetrohydrofuran (150mL). 2,2,6,6-tetramethylpiperidine (5 drops) was added and the mixture was heated at 60°C for 5h. The solvent was removed under reduced pressure. The product was then chromatographed on 8% deactivated alumina (120g) with a dichloromethane/hexane gradient (25:75 to 100% CH_2Cl_2). Unreacted **10** was obtained in the early fractions eluted with 50% dichloromethane; later fractions (100% CH_2Cl_2) contained the product. Washing the column with a 1% methanol dichloromethane mixture gave the product of double addition. The combined product containing fractions were evaporated to give **12** as a clear oil (1.4g, 2.5mmol,18%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.2 (s, 2H, *cis* C***H***=C***H***), 4.3 (m, 8H, $CO_2C\boldsymbol{H}_2$), 3.7 (m, 19H, $C\boldsymbol{H}_2O$, $C\boldsymbol{H}_2OH$, SC***H***), 2.9-2.6 (m, 4H, $C\boldsymbol{H}_2CHS$, $SC\boldsymbol{H}_2$), 2.0 (s, 1H, O***H***), 1.8 (m, 2H, $C\boldsymbol{H}_2CH_2OH$) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 26) ; MS (CI, m/e): 553(M+1) ; Analysis calculated for $C_{23}H_{36}O_{13}S$: C 49.99%; H 6.57%; S 5.80%; Found C 49.75%; H 6.52%; S 5.87%

3-(4-methanesulphonyl-1-thiabutyl)-1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (16)



Compound **12** (1.4g, 2.5mmol) and triethyl amine (2.02g, 20mmol, 8eq) were dissolved in dichloromethane (150mL) and the mixture cooled to -10°C. Methane sulphonyl chloride (860mg, 7.5mmol, 3eq) in dichloromethane (5mL) was added dropwise over 30 minutes. The reaction was allowed to warm to room temperature and was stirred for a further 2h. The reaction mixture was then washed with saturated sodium chloride (2x100mL), 10% hydrochloric acid (2x100mL), 10% sodium bicarbonate (2x100mL) and again with saturated sodium chloride (2x100mL), dried over sodium sulphate and the solvent removed to give **16** as a yellow oil (1.23g, 2.0mmol, 80%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.2 (s, 2H, *cis* C***H***=C***H***), 4.2 (m, 10H, CO_2CH_2, CH_2OMs), 3.6 (m, 17H, CH_2O, SC***H***), 2.8-2.6 (m, 4H, CH_2CHS, SCH_2), 2.1 (m, 2H, CH_2CH_2OMs) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 6)

3-(4-iodo-1-thiabutyl)-1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-ene (17)

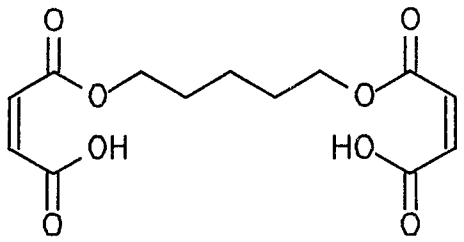


Compound **16** (1.23g, 2.0mmol) and sodium iodide (3g, 20mmol, 10eq) were mixed in acetone (100mL). The mixture was heated at reflux overnight. The solvent was removed under reduced pressure. The reaction mixture was dissolved in dichloromethane (200mL), washed with water (2x100mL), dried over sodium sulphate, and the solvent removed under reduced pressure, to give **17** as a yellow oil (1g, 1.5mmol, 75%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.2 (s, 2H, *cis* C*H*–C*H*), 4.2 (m, 8H, CO_2CH_2), 3.6 (m, 17H, CH_2O, SC*H*), 3.2 (t, J=6Hz, 2H, CH_2I), 3.0-2.6 (m, 4H, CH_2CHS, SCH_2), 2.0 (m, 2H, CH_2CH_2I) ; ^{13}CNMR 62.89 ($CDCl_3$) (Refer to Table 26) ; MS (CI, m/e): 663(M+1) ; Analysis calculated for $C_{23}H_{35}O_{12}SI$: C 41.70%; H 5.33%; S 4.84%; Found C 42.36%; H 5.30%; S 5.16%

Table 26. ^{13}CNMR Data for compounds **12, 16** and **17** (ppm)

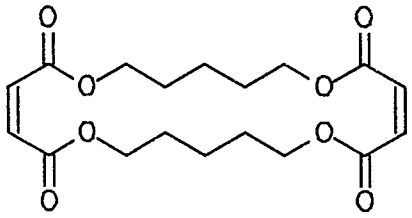
Assignment	12	16	17
C=O	171.4	171.1	171.1
	170.2	170.0	170.0
	165.0	165.0	165.0
cis ***C***=***C***	129.7	129.7	129.7
Ether	70.5	70.5	70.6
$\boldsymbol{C}H_2O$	68.9	68.7	68.9
	68.8		68.8
$CO_2\boldsymbol{C}H_2$	64.4	64.5	64.5
	64.3	64.3	64.3
	64.0	64.0	64.0
$\boldsymbol{C}H_2$	41.7	41.6	41.6
	36.4	36.3	36.4
$\boldsymbol{C}H_3SO_3$		37.3	
$\boldsymbol{C}H_2X$	61.0	67.8	4.5
$\boldsymbol{C}H_2S$	31.8	28.5	32.0
$\boldsymbol{C}H_2CH_2$	28.1	27.3	32.3

5,11-dioxa-4,12-dioxopentadeca-2,13-diene-1,15-dicarboxylic acid (18)



The 1,5-pentanediol (104g, 1mol) and maleic anhydride (196g, 2mol, 2eq) were mixed in benzene (500mL) and refluxed for 6h. The benzene was removed under reduced pressure to give **18** as a colourless solid (300g, 1mol, quantitative): mp 77-81°C; ^{1}HNMR 90MHz (δ, $CDCl_3$): 6.3 (s, 4H, *cis* C***H***=C***H***), 4.2 (t, J=6Hz, 8H, $CO_2C\boldsymbol{H}_2$), 1.6 (m, 6H, C$\boldsymbol{H}_2$); MS (CI, m/e): 301(M+1)

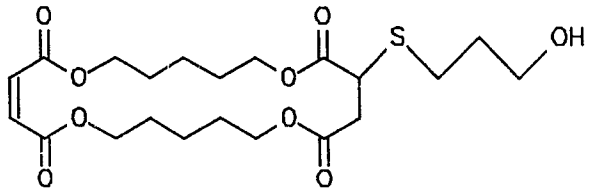
1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosa-3,14-diene (19)



Compound **18** (30g, 0.1mol) and 1,5-pentanediol (10.4g, 0.1mol, 1eq) were mixed in benzene (1500mL). Methanesulphonic acid (15 drops) was added and the mixture was heated with azeotropic removal of water (Dean-Stark) for 12h. The solvent was removed under reduced pressure. The solid was triturated with diethylether (200mL), then recrystallised three times from ethyl acetate, to give **19** as a colourless crystalline solid (3.8g, 10mmol, 10%): mp 130-131°C;

^{1}HNMR 90MHz ($CDCL_3$) 6.2 (s, 4H, 2x*CH*=*CH*), 4.2 (t, J=6Hz, 8H, CH_2O), 1.8-1.3 (m, 12H, 6x*CH*2); ^{13}CNMR 62.89MHz (δ, $CDCl_3$) 165.4 (4x*C*=O), 129.8 (2x*C*H=*C*H), 65.0 (4xCO_2CH_2), 28.1 (CH_2), 22.0 (CH_2); MS (CI, m/e): 369(M+1); Analysis calculated for $C_{16}H_{22}O_4$: C 58.69%; H 6.57%; Found C 58.73%; H 6.57%

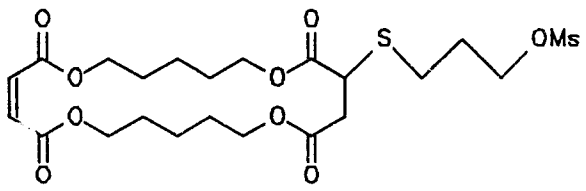
3-(4-hydroxy-1-thiabutyl)-1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosa-14-ene (20)



Compound **19** (5g, 14mmol) and 3-mercapto-1-propanol (1.3g, 14mmol, 1eq) were added to 2-propanol (200mL). Piperidine (10 drops) was added and the mixture was heated at reflux for 1h 45min. The solvent was removed under reduced pressure. The product was then chromatographed on 8% deactivated alumina (120g) with a dichloromethane/hexane gradient (50:50 to 100% CH_2Cl_2). Unreacted **19** was obtained in the early fractions eluted with 50% dichloromethane; later fractions (100%CH_2Cl_2) contained the product. Washing the column with a 1% methanol dichloromethane mixture yielded the product of double addition. The combined product containing fractions were evaporated to give **20** as a clear oil (960mg, 2.0mmol, 14%): ^{1}HNMR 360MHz

(δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, $CO_2C\boldsymbol{H}_2$), 3.7 (m, 3H, $C\boldsymbol{H}_2OH$, SC***H***), 2.9-2.6 (m, 4H, $C\boldsymbol{H}_2CHS$, $SC\boldsymbol{H}_2$), 2.0(s, 1H, O***H***), 1.8 (m, 2H, $C\boldsymbol{H}_2CH_2OH$), 1.7-1.5 (m, 12H, $C\boldsymbol{H}_2$) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 27) ; MS (CI, m/e): 461(M+1) ; Analysis calculated for $C_{21}H_{32}O_9S$: C 54.77%; H 7.00%; S 6.96%; Found C 54.83%; H 6.94%; S 7.35%

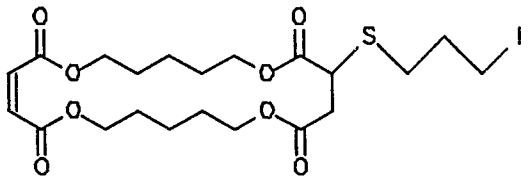
3-(4-methanesulphonyl-1-thiabutyl)-1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosa-14-ene (22)



Compound **20** (960mg, 2mmol) and triethyl amine (2.02g, 20mmol, 10eq) were dissolved in dichloromethane (200mL) and the mixture cooled to -10°C. Methane sulphonyl chloride (860mg, 7.5mmol, 3.75eq) in dichloromethane (5mL) was added dropwise over 30 minutes. The reaction was allowed to warm to room temperature and was stirred for a further 2h. The reaction mixture was then washed with saturated sodium chloride (2x100mL), 10% hydrochloric acid (2x100mL), 10% sodium bicarbonate (2x100mL) and again with saturated sodium chloride (2x100mL), dried over sodium sulphate and the solvent removed to give **22** as a yellow oil (900mg, 1.7mmol, 85%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 10H, $CO_2C\boldsymbol{H}_2$, $C\boldsymbol{H}_2OMs$), 3.6 (d of d J=3Hz and 6Hz, 1H, SC***H***), 2.9-2.6 (m, 4H, $C\boldsymbol{H}_2CHS$, $SC\boldsymbol{H}_2$), 2.0 (m,

2H, C***H***$_2$CH$_2$OMs), 1.8-1.4 (m, 12H, C***H***$_2$) ; ^{13}CNMR 90.57 ($CDCl_3$) (Refer to Table 27)

3-(4-iodo-1-thiabutyl)-1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosa-14-ene (23)



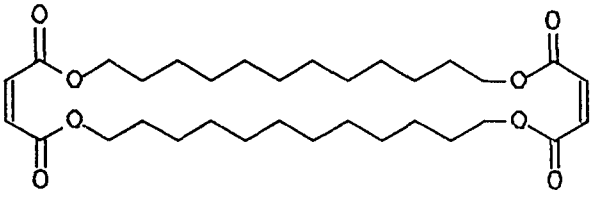
Compound **22** (900mg, 1.7mmol) and sodium iodide (3g, 20mmol, 12eq) were mixed in acetone (200mL). The mixture was heated at reflux overnight. The solvent was removed under reduced pressure. The reaction mixture was dissolved in dichloromethane (200mL), washed with water (2x100mL), dried over sodium sulphate, and the solvent removed under reduced pressure, to give **23** as a yellow oil (540mg, 940µmol, 55%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.7 (s, 2H, *trans* C***H***=C***H***), 4.1 (m, 8H, CO_2C***H***$_2$), 3.5 (d of d J=3Hz and 6Hz, 1H, SC***H***), 3.1 (t, 2H, C***H***$_2$I), 2.8-2.5 (m, 4H, C***H***$_2$CHS, SC***H***$_2$), 1.9 (m, 2H, C***H***$_2$CH$_2$I), 1.7-1.4 (m, 12H, C***H***$_2$) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 27) ; MS (CI, m/e): 571(M+1) ; Analysis calculated for $C_{21}H_{31}O_8SI$: C 44.22%; H 5.48%; S 5.62%; I 22.25%; Found C 45.00%; H 5.55%; S 6.24%; I 21.69%

Table 27. ^{13}CNMR Data for compounds **20**, **22** and **23** (ppm)

Table 27 Assignment	**20**	**22**	**23**
***C*=O**	171.3	170.9	170.8
	170.3	169.9	169.8
	165.0	164.6	164.6
	164.9		
trans ***C*=*C***	133.4	133.2	133.2
CO_2CH_2	65.0	64.8	64.8
	64.9	64.7	64.7
	64.6	64.3	64.3
CH_2CHS	42.5	42.2	42.1
CH_2CHS	36.0	35.7	35.8
CH_2	28.4	28.2	28.2
	28.3	28.1	28.0
	23.2	22.9	22.9
	22.9	22.8	22.8
CH_3SO_3		37.1	

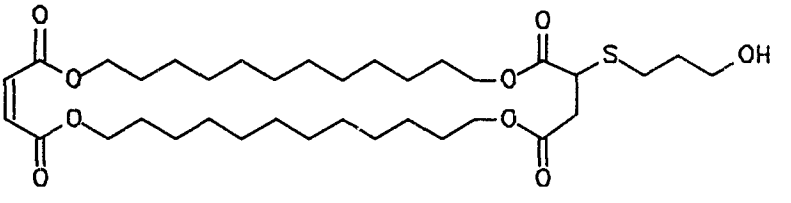
Table 27 Assignment	20	22	23
CH_2X	60.6	67.6	4.5
CH_2S	31.4	27.9	31.8
CH_2CH_2X	27.9	27.2	31.8

1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatriconta-3,21-diene (24)



Compound **24** was prepared as descibed by Fuhrhop[31]. (**Scheme 15**) Physical and spectral properties were identical to those reported.

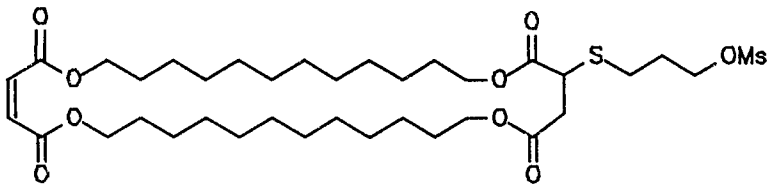
3-(4-hydroxy-1-thiabutyl)-1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatriconta-21-ene (26)



Compound **24** (5.5g, 9.8mmol) and 3-mercapto-1-propanol (900mg, 9.8mmol, 1eq) were added to 2-propanol (150mL). Piperidine (10 drops) was added and the mixture was heated at reflux for 1h 45min. The solvent was

removed under reduced pressure. The product was then chromatographed on 8% deactivated alumina (100g) with a dichloromethane/hexane gradient (50:50 to 100% CH_2Cl_2). Unreacted **24** was obtained in the early fractions eluted with 50% dichloromethane; later fractions (100%CH_2Cl_2) contained the product. Washing the column with a 1% methanol dichloromethane mixture yielded the product of double addition. The combined product containing fractions were evaporated to give **26** as a colourless solid (720mg, 1.1mmol, 11%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, $CO_2C\boldsymbol{H}_2$), 3.7 (m, 3H, $C\boldsymbol{H}_2OH$, SC***H***), 3.0-2.6 (m, 4H, $C\boldsymbol{H}_2CHS$, $SC\boldsymbol{H}_2$), 1.8 (m, 2H, $C\boldsymbol{H}_2CH_2OH$), 1.5-1.2 (m, 41H, $C\boldsymbol{H}_2$, O***H***) ; ^{13}CNMR 62.89 ($CDCl_3$) (Refer to Table 28) ; MS (CI, m/e): 657(M+1) ; Analysis calculated for $C_{34}H_{60}O_9S$: C 63.99%; H 9.21%; S 4.88%; Found C 64.23%; H 9.08%; S 5.26%

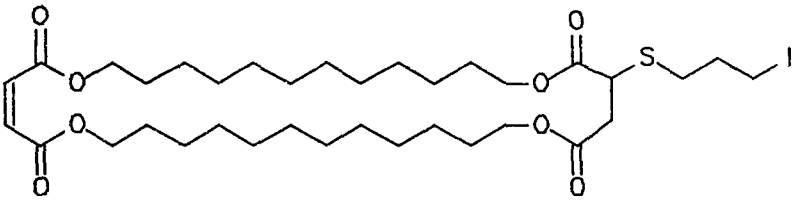
3-(4-methanesulphonyl-1-thiabutyl)-1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatriconta-21-ene (27)



Compound **26** (720mg, 1.1mmol) and triethyl amine (2.02g, 20mmol, 18eq) were dissolved in dichloromethane (50mL) and the mixture cooled to -10°C. Methane sulphonyl chloride (860mg, 7.5mmol, 7eq) in dichloromethane (5mL) was added dropwise over 30 minutes. The reaction was allowed to

warm to room temperature and was stirred for a further 2h. The reaction mixture was then washed with saturated sodium chloride (2x100mL), 10% hydrochloric acid (2x100mL), 10% sodium bicarbonate (2x100mL) and again with saturated sodium chloride (2x100mL), dried over sodium sulphate and the solvent removed to give **27** as a yellow semi-solid (740mg, 1.0mmol, 90%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, $CO_2C\boldsymbol{H}_2$), 3.6 (d of d, 3Hz and 6Hz, SCH), 3.0 (s, 3H, $C\boldsymbol{H}_2OMs$), 3.2-2.6 (m, 4H, $C\boldsymbol{H}_2CHS$, $SC\boldsymbol{H}_2$), 2.0 (m, 2H, $C\boldsymbol{H}_2CH_2OMs$), 1.7-1.1 (m, 40H, $C\boldsymbol{H}_2$) ; ^{13}CNMR 62.89 ($CDCl_3$) (Refer to Table 28)

3-(4-iodo-1-thiabutyl)-1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclo-hexatriconta-21-ene (28)



Compound **27** (740mg, 1.0mmol) and sodium iodide (3g, 20mmol, 20eq) were mixed in acetone (100mL). The mixture was heated at reflux overnight. The solvent was removed under reduced pressure. The reaction mixture was dissolved in dichloromethane (200mL), washed with water (2x100mL), dried over sodium sulphate, and the solvent removed under reduced pressure, to give **28** as a yellow semi-solid (640mg, 830μmol, 83%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 2H, *trans* C***H***=C***H***), 4.2 (m, 8H, $CO_2C\boldsymbol{H}_2$), 3.6 (d of d J=3Hz and 6Hz,

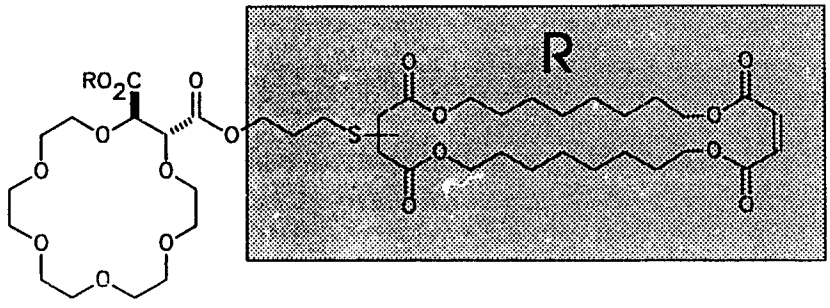
1H, SC***H***), 3.2 (t, 2H, C***H***$_2$I), 3.0-2.5 (m, 4H, C***H***$_2$CHS, SC***H***$_2$), 2.1 (m, 2H, C***H***$_2$CH$_2$I), 1.7-1.1 (m, 40H, C***H***$_2$) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 28) ; MS (CI, m/e): 767(M+1) ; Analysis calculated for $C_{35}H_{59}O_8SI$: C 54.82%; H 7.56%; S 4.18%; I 16.55%; Found C 54.98%; H 7.88%; S 4.76; I 16.88%

Table 28. ^{13}CNMR Data for compounds **26**, **27** and **28** (ppm)

Table 28 Assignment	**26**	**27**	**28**
C=O	171.3	171.3	171.3
	170.4	170.4	170.4
	165.0	165.0	165.0
trans ***C***=***C***	133.6	133.5	133.5
CO_2***C***H$_2$	65.4	65.6	65.6
	65.1	65.4	65.4
		65.1	65.1
CH$_2$***C***HS	41.8	41.7	41.6
CH$_2$CHS	36.6	36.5	36.6

Table 28 Assignment	26	27	28
CH_2	29.2	29.2	29.3
	29.0	29.0	29.0
	28.4	28.4	28.5
	25.9	25.9	25.9
	25.6	25.6	25.6
CH_3SO_3		37.4	
CH_2X	61.1	67.8	4.4
CH_2S	31.7	28.4	32.0
CH_2CH_2X	28.0	27.4	32.4

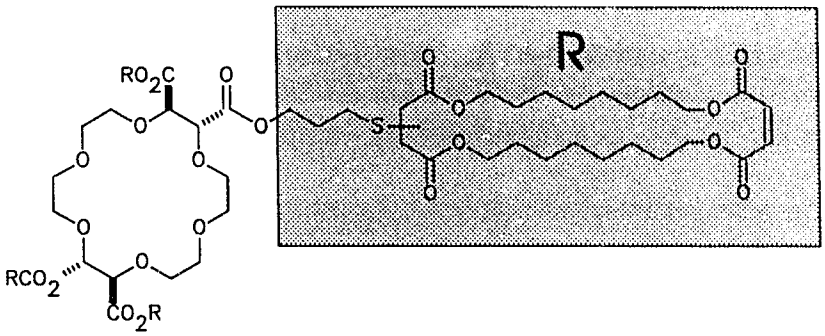
Bis(3-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (29)



To a stirred solution of 2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylic acid (138mg, 390µmol) in methyl sulphoxide (20mL) at 60°C under argon, tetramethylammonium hydroxide (140mg, 817µmol, 1eq per CO_2H) was added. To this solution compound **6** (1g, 1.53mmol, 2eq per CO_2H) was added as a solution in methyl sulphoxide (2mL), and the mixture stirred for a further 4h. The resulting amber solution was evaporated to dryness, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol , later fractions contained **3**, the product containing fractions were combined and the solvent removed to give **29** as a yellow oil (200mg, 141µmol, 36%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 4H, 2x*trans* C***H***=C***H***), 4.4 (s, 2H, 2xC***H***O *methine*), 4.2-4.0 (m, 20H, 8x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 2x$CO_2C\boldsymbol{H}_2$ *crown*), 3.7 (m, 2H, 2xSC***H***), 3.6 (br.m, 20H 10x$C\boldsymbol{H}_2O$), 3.0-2.5 (m, 8H, 2x$C\boldsymbol{H}_2$CHS, 2x$SC\boldsymbol{H}_2$), 1.9 (m, 4H, 2x$C\boldsymbol{H}_2CH_2OR$), 1.5-1.2 (m, 48H, 24x$C\boldsymbol{H}_2$)

; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 29) ; Analysis calculated for $C_{68}H_{108}O_{26}S_2$: C 58.10%; H 7.74%; 4.56%; Analysis calculated with 1% DMSO : C 57.83%; H 7.74%; S4.97%; Found C 57.84%; H 7.52%; S 4.97%

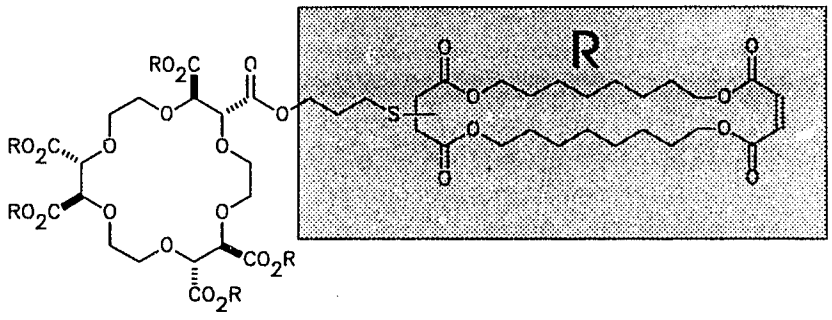
Tetrakis(3-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (30)



To a stirred solution of 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxa-cyclooctadecane-2,3,11,12-tetracarboxylic acid (48mg, 110µmol) in methyl sulphoxide (20mL) at 60°C under argon, tetramethylammonium hydroxide (91mg, 502µmol, 1.1eq per. CO_2H) was added. To the resulting milky solution compound **6** (600mg, 917µmol, 2eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **3**, the product containing

fractions were combined and the solvent removed to give **30** as a yellow oil (205mg, 78μmol, 71%): ^{1}HNMR 360MHz (δ, CD_2Cl_2): 6.8 (s, 8H, 4x*trans* C***H***=C***H***), 4.3 (s, 4H, 4xC***H***O *methine*), 4.2-4.0 (m, 40H, 16x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 4x$CO_2C\boldsymbol{H}_2$ *crown*), 3.8 (m, 4H, 4xSC***H***), 3.6 (br.m, 16H 8xC$\boldsymbol{H}_2$O), 3.0-2.6 (m, 16H, 4xC$\boldsymbol{H}_2$CHS, 4xSC$\boldsymbol{H}_2$), 2.0 (m, 8H, 4xC$\boldsymbol{H}_2$$CH_2$OR), 1.6-1.2 (br.m, 96H, 48xC$\boldsymbol{H}_2$) ; ^{13}CNMR 90.57MHz (CD_2Cl_2) (Refer to Table 29)

Hexakis(3-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (31)



To a stirred solution of 2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxa-cyclooctadecane-2,3,8,9,14,15-hexacarboxylic acid (40mg, 76μmol) in methyl sulphoxide (20mL) at 60°C under argon, tetramethylammonium hydroxide (83mg, 458μmol, 1.5eq per. CO_2H) was added. To the resulting solution compound **6** (603mg, 920μmol, 3eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol

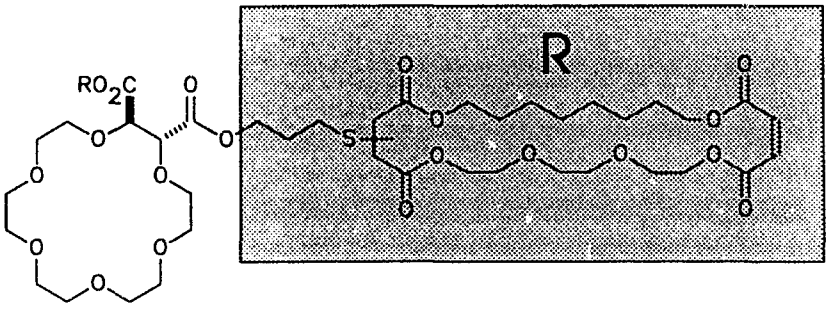
(2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **3**, the product containing fractions were combined and the solvent removed to give **30** as a yellow oil (151mg, 41μmol, 53%): ^{1}HNMR 360MHz (δ, CD_2Cl_2): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 12H, 6x*trans* C***H***=C***H***), 4.4 (s, 6H, 6xC***H***O *methine*), 4.3-3.9 (m, 60H, 24xCO_2C***H***$_2$ *macrocycle*, 6xCO_2C***H***$_2$ *crown*), 3.7 (m, 6H, 6xSC***H***), 3.6 (br.s, 12H 6xC***H***$_2$O), 3.0-2.5 (m, 24H, 6xC***H***$_2$CHS, 6xSC***H***$_2$), 1.9 (m, 12H, 6xC***H***$_2$$CH_2$OR), 1.7-1.1 (br.m, 144H, 72xC***H***$_2$) ; ^{13}CNMR 90.57MHz (CD_2Cl_2) (Refer to Table 29) ; Analysis calculated for $C_{180}H_{276}O_{66}S_6$: C 58.61%; H 7.54%; S 5.22%; Analysis calculated with 1% DMSO : C 58.33%; H 7.54%; S 5.63%; Found C 58.05%; H 7.38%; S 5.80%

Table 29. ^{13}CNMR Data for compounds **29**, **30** and **31** (ppm)

Table 29 Assignment	**29**	**30**	**31**
C=O	171.4	171.7	171.7
	170.4	170.8	170.8
	164.8	165.2	165.2
Crown	169.2	169.5	169.4
trans ***C***=***C***	133.4	133.8	133.7
Crown ***C***HO	79.6	80.3	80.2
Crown ***C***H_2O	71.0	71.5	70.9
	70.6	70.5	
	70.4		
	70.3		
Macrocycle	65.2	65.6	65.6
CO_2***C***H_2	64.8	65.5	65.5
		65.3	65.1
		65.2	
Crown	63.4	63.9	63.9
CO_2***C***H_2			

Table 29 Assignment	**29**	**30**	**31**
$CH_2\mathbf{C}HS$	41.4	41.9	41.8
$\mathbf{C}H_2CHS$	36.4	36.9	36.9
$\mathbf{C}H_2$	28.8	29.8	29.3
	28.7	29.2	29.3
	28.4	28.8	29.2
	28.3	28.7	28.7
	25.9	28.7	26.4
	25.2	26.4	25.7
		25.7	25.6
		25.6	
Propyl $\mathbf{C}H_2$	28.1	28.6	28.6
	27.8	28.2	28.2

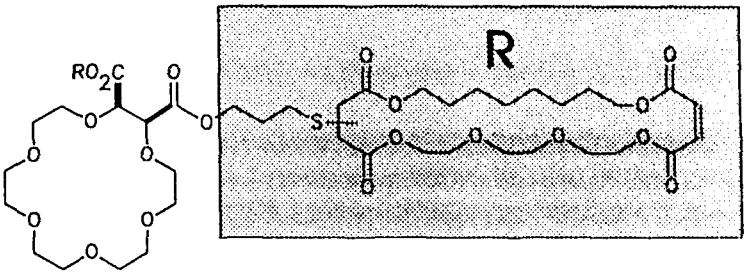
Bis(3-and 4-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (32)



To a stirred solution of 2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylic acid (67mg, 190µmol) in methyl sulphoxide (20mL) at 70°C under argon, tetramethylammonium hydroxide (73mg, 400µmol, 1.1 eq per. CO_2H) was added. To this solution compound **11** (500mg, 760µmol, 2eq per. CO_2H) was added as a solution in methyl sulphoxide (2mL), and the mixture stirred for a further 12h. The resulting amber solution was evaporated to dryness, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **8**, the product containing fractions were combined and the solvent removed to give **32** as a yellow oil (180mg, 128µmol, 67%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 4H, 2x*trans* C***H***=C***H***), 4.3 (s, 2H, 2xC***H***O *methine*), 4.2-3.9 (m, 20H, 8x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 2x$CO_2C\boldsymbol{H}_2$ *crown*), 3.8 (m, 2H, 2xSC***H***), 3.6 (br.m, 36H, 10x$C\boldsymbol{H}_2O$ *crown*, 8x$C\boldsymbol{H}_2O$ *macrocycle*), 3.0-2.5 (m, 8H, 2x$C\boldsymbol{H}_2$CHS, 2xS$C\boldsymbol{H}_2$), 1.9 (m, 4H,

2x$CH_2$$CH_2$OR), 1.7-1.2 (br.m, 24H, 12xCH_2) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 30) ; Analysis calculated for $C_{64}H_{100}O_{30}S_2$: C 54.38%; H 7.13%; S 4.54%; Anaalysis calculated with 1% DMSO : C 54.15%; H 7.13%; S 4.95%; Found C 53.94%; H 7.13%; S 5.43%

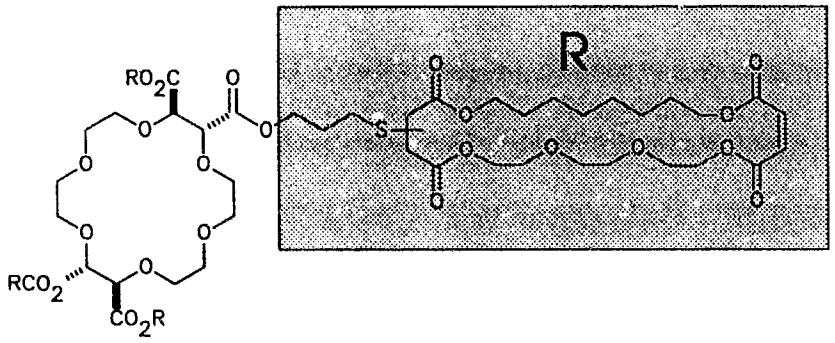
Bis(3-and 4-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3S-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (33)



To a stirred solution of 2R,3S-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylic acid (67mg, 190μmol) in methyl sulphoxide (20mL) at 70°C under argon, tetramethylammonium hydroxide (68.8mg, 380μmol, 1 eq per. CO_2H) was added. To this solution compound **11** (500mg, 760μmol, 2eq per. CO_2H) was added as a solution in methyl sulphoxide (2mL), and the mixture stirred for a further 12h. The resulting amber solution was evaporated to dryness, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **8**, the product containing fractions were combined and the solvent removed to give **33** as a

yellow oil (170mg, 120µmol, 63%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 4H, 2x*trans* C***H***=C***H***), 4.6 (s, 2H, 2xC***H***O *methine*), 4.3-3.9 (m, 20H, 8x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 2x$CO_2C\boldsymbol{H}_2$ *crown*), 3.8 (m, 2H, 2xSC***H***), 3.6 (br.m, 36H, 10x$C\boldsymbol{H}_2O$ *crown*, 8x$C\boldsymbol{H}_2O$ *macrocycle*), 3.0-2.5 (m, 8H, 2x$C\boldsymbol{H}_2$CHS, 2xS$C\boldsymbol{H}_2$), 1.9 (m, 4H, 2x$C\boldsymbol{H}_2CH_2OR$), 1.7-1.1 (br.m, 24H, 12x$C\boldsymbol{H}_2$) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 30) ; Analysis calculated for $C_{64}H_{100}O_{30}S_2$: C 54.38%; H 7.13%; S 4.54%; Analysis calculated with 1% DMSO : C 54.15%; H 7.13%; S 4.95%; Found C 53.86%; H 6.78%; S 5.33%

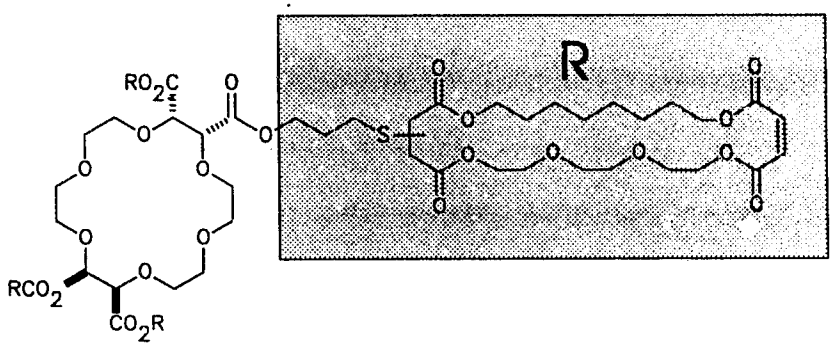
Tetrakis(3-and 4-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (34)



To a stirred solution of 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxa-cyclooctadecane-2,3,11,12-tetracarboxylic acid (83.6mg, 190µmol) in methyl sulphoxide (20mL) at 70°C under argon, tetramethylammonium hydroxide (150mg, 828µmol, 1.1eq per. CO_2H) was added. To the resulting milky solution compound **11** (1g, 1.52mmol, 2eq per. CO_2H) was added as a solution (5mL,

methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **8**, the product containing fractions were combined and the solvent removed to give **34** as a yellow oil (320mg, 125μmol, 66%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 8H, 4x*trans* C***H***=C***H***), 4.4-4.0 (br.m, 44H, 16x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 4x$CO_2C\boldsymbol{H}_2$ *crown*, 4xC***H***O *methine*), 3.7-3.5 (br.m, 52H, 8x$C\boldsymbol{H}_2O$ *crown*, 16x$C\boldsymbol{H}_2O$ *macrocycle*, 4xSC***H***), 3.0-2.5 (m, 16H, 4x$C\boldsymbol{H}_2$CHS, 4xS$C\boldsymbol{H}_2$), 1.9 (m, 8H, 4x$C\boldsymbol{H}_2CH_2$OR), 1.7-1.2 (br.m, 48H, 24x$C\boldsymbol{H}_2$) ; ^{13}CNMR 90.57MHz (CD_2Cl_2) (Refer to Table 30)

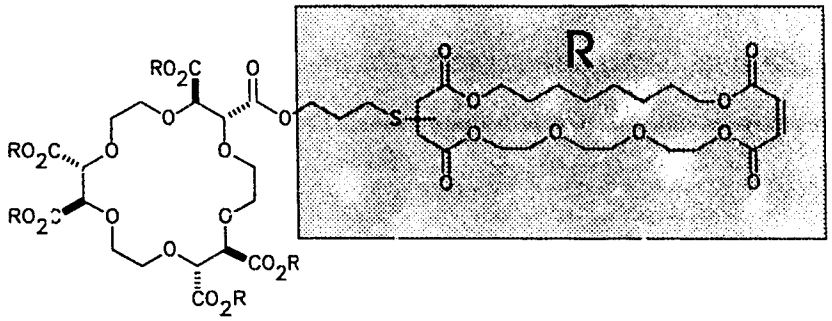
Tetrakis(3-and 4-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3S,11R,12S-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (35)



To a stirred solution of 2R,3S,11R,12S-1,4,7,10,13,16-hexaoxa-cyclooctadecane-2,3,11,12-tetracarboxylic acid (42mg, 95μmol) in methyl sulphoxide (20mL) at

70°C under argon, tetramethylammonium hydroxide (75mg, 414μmol, 1.1eq per. CO_2H) was added. To the resulting solution compound **11** (500mg, 760μmol, 2eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **3**, the product containing fractions were combined and the solvent removed to give **35** as a yellow oil (160mg, 62μmol, 65%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 8H, 4x*trans* C***H***=C***H***), 4.5 (s, 4xC***H***O *methine*), 4.3-4.0 (br.m, 40H, 16xCO_2C***H***$_2$ *macrocycle*, 4xCO_2C***H***$_2$ *crown*), 3.7-3.5 (br.m, 52H, 8xC***H***$_2$O *crown*, 16xC***H***$_2$O *macrocycle*, 4xSC***H***), 3.0-2.5 (m, 16H, 4xC***H***$_2$CHS, 4xSC***H***$_2$), 1.9 (m, 8H, 4xC***H***$_2$$CH_2$OR), 1.7-1.2 (br.m, 48H, 24xC***H***$_2$) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 30) ; Analysis calculated for $C_{116}H_{176}O_{54}S_4$: C 54.36%; H 6.92%; S 5.00%; Analysis calculated with 1% DMSO : C 54.12%; H 6.92%; S 5.41%; Found C 54.15%; H 7.00%; S 5.86%

Hexakis(3-and 4-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (36)



To a stirred solution of 2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxa-cyclooctadecane-2,3,8,9,14,15-hexacarboxylic acid (66mg, 125µmol) in methyl sulphoxide (20mL) at 70°C under argon, tetramethylammonium hydroxide (150mg, 829µmol, 1.1eq per. CO_2H) was added. To the resulting solution compound **11** (1g, 1.52mmol, 2eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **8**, the product containing fractions were combined and the solvent removed to give **36** as a yellow oil (151mg, 41µmol, 53%): ^{1}HNMR 360MHz (δ, CD_2Cl_2): 6.8 (s, 12H, 6x*trans* C***H***=C***H***), 4.4-4.0 (br.m, 66H, 24x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 6x$CO_2C\boldsymbol{H}_2$ *crown*, 6xC***H***O *methine*), 3.7-3.5 (br.m, 66H, 6x$C\boldsymbol{H}_2C$ *crown*, 24x$C\boldsymbol{H}_2O$ *macrocycle*, 6xSC***H***),

3.0-2.5 (m, 24H, 6xC***H***$_2$CHS, 6xSC***H***$_2$), 1.9 (m, 12H, 6xC***H***$_2$CH$_2$OR), 1.7-1.2 (br.m, 72H, 36xC***H***$_2$) ; ^{13}CNMR 90.57MHz (CD_2Cl_2) (Refer to Table 30)

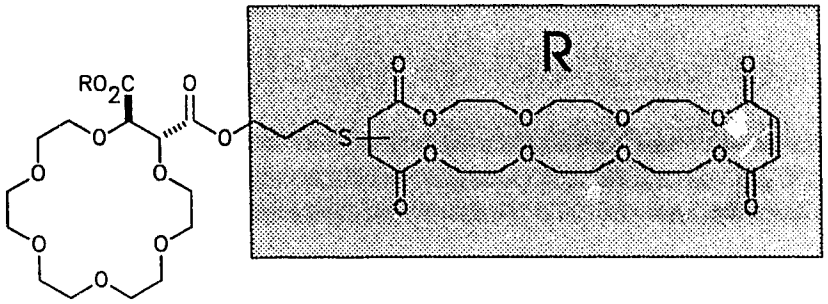
Table 30. ^{13}CNMR Data for compounds **32, 33, 34, 35** and **36** (ppm)

Table 30 Assignment	**32**	**33**	**34**	**35**	**36**
C=O	171.3	171.2	171.8	171.3	171.6
	170.4	171.1	171.6	170.4	170.8
	170.3	170.2	170.8	164.8	165.2
	164.8	170.1	170.6		165.1
	164.7	164.6	165.2		
		164.5	165.1		
Crown	169.3	169.0	169.5	168.9	169.4
trans ***C=C***	134.0	133.7	134.3	134.0	134.3
	133.1	132.8	133.3	133.1	133.3
Crown ***C***HO	79.6	80.2	80.1	80.3	80.2
				80.0	

Table 30 Assignment	**32**	**33**	**34**	**35**	**36**
Macrocycle	71.0	70.7	71.0	72.5	71.1
$\mathbf{C}H_2O$	70.8	70.6	70.8	70.8	71.0
	70.7	70.5	69.3	70.7	70.8
	70.6	70.4		70.4	69.3
	70.4	70.3		70.3	69.2
	70.3	70.2		68.9	
	69.0	68.7			
	68.9				
Crown	Hidden	Hidden	71.5	Hidden	Hidden
$\mathbf{C}H_2O$			70.5		
Macrocycle	65.3	65.0	65.6	65.3	65.6
$CO_2\mathbf{C}H_2$	65.2	64.9	65.5	65.2	65.5
	64.8	64.5	64.9	64.8	65.1
	64.4	65.2	64.8	64.4	64.9
	64.3	64.1	64.3	64.3	64.8
	64.2	64.0		63.9	
	63.8	63.6			
Crown	63.4	63.2	64.0	63.6	63.9
$CO_2\mathbf{C}H_2$				63.5	

Table 30 Assignment	**32**	**33**	**34**	**35**	**36**
CH_2CHS	41.5	41.3	42.0	41.5	42.0
	41.4	41.1	41.8	41.4	41.7
CH_2CHS	36.5	36.3	36.9	36.5	36.9
	36.3	36.1	36.7	36.4	36.7
CH_2	28.7	28.5	29.2	28.7	29.2
	28.6	28.4	29.1	28.6	29.0
	28.4	28.3	29.0	28.4	28.9
	28.3	28.1	28.9	28.3	28.7
	28.1	28.0	28.8	28.2	28.6
	27.9	27.7	28.7	27.9	28.5
	27.8	27.5	28.6	27.8	28.3
	25.8	25.5	28.5	25.7	28.0
	25.7	25.4	28.3	25.3	26.2
	25.3	25.0	26.1	25.2	25.8
	25.2	24.9	25.7		25.6
			25.6		

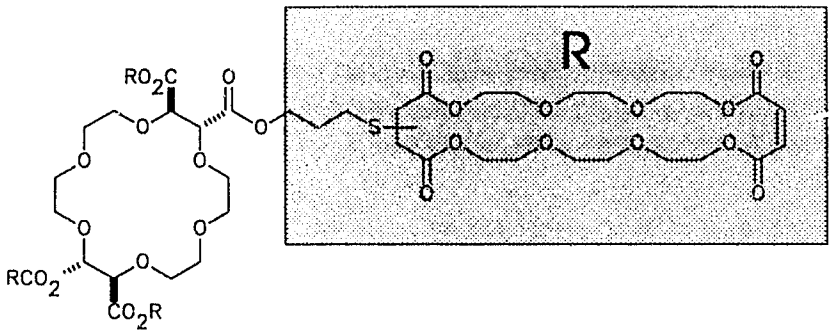
Bis(3-(1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (37)



To a stirred solution of 2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylic acid (73.5mg, 209μmol) in methyl sulphoxide (20mL) at 68°C under argon, tetramethylammonium hydroxide (88mg, 486μmol, 1.2 eq per. CO_2H) was added. To this solution compound **17** (320mg, 483μmol, 1.2eq per. CO_2H) was added as a solution in methyl sulphoxide (2mL), and the mixture stirred for a further 12h. The resulting amber solution was evaporated to dryness, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol , later fractions contained **12**, the product containing fractions were combined and the solvent removed to give **37** as a yellow oil (140mg, 98μmol, 47%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.2 (s, 4H, 2x*cis* C***H***=C***H***), 4.4 (s, 2xC***H***O *methine*), 4.3-4.0 (m, 20H, 8xCO_2C***H***$_2$ *macrocycle*, 2xCO_2C***H***$_2$ *crown*), 3.8 (m, 2H, 2xSC***H***), 3.7-3.6 (br.m, 52H, 10xC***H***$_2$O *crown*, 16xC***H***$_2$O *macrocycle*), 3.0-2.6 (m, 8H, 2xC***H***$_2$CHS, 2xSC***H***$_2$), 1.9 (m, 4H,

2xCH_2CH_2OR) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 31) ; Analysis calculated for $C_{60}H_{92}O_{34}S_2$: C 50.7%; H 6.52%; S 4.51; Analysis calculated with 1% DMSO : C 50.50%; H 6.52%; S 4.92%; Found C 50.00%; H 6.43%; S 5.02%

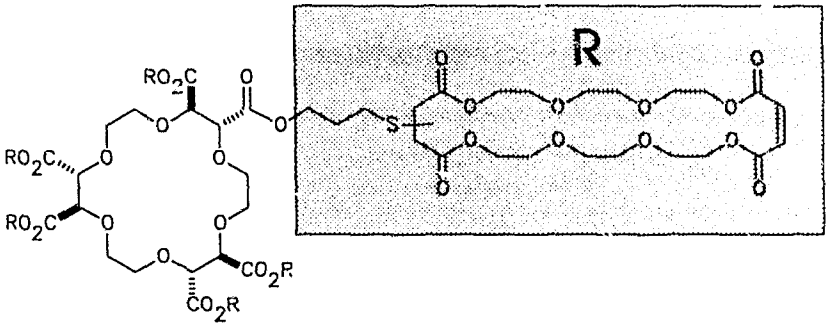
Tetrakis(3-(1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (38)



To a stirred solution of 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylic acid (29mg, 66µmol) in methyl sulphoxide (20mL) at 68°C under argon, tetramethylammonium hydroxide (50mg, 280µmol, 1.1eq per. CO_2H) was added. To the resulting milky solution compound **17** (200mg, 302µmmol,1.1eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **12**, the product containing

fractions were combined and the solvent removed to give **38** as a yellow oil (95mg, 37µmol, 56%): ^{1}HNMR 360MHz (δ, CD_2Cl_2): 6.3 (s, 8H, 4x*cis* C***H***=C***H***), 4.4-4.1 (m, 44H, 16xCO_2CH_2 *macrocycle*, 4xCO_2CH_2 *crown* 4xC***H***O *methine*), 3.8 (m, 4H, 4xSC***H***), 3.7-3.5 (br.m, 80H, 8xCH_2O *crown*, 32xCH_2O *macrocycle*), 3.0-2.6 (m, 16H, 4xCH_2CHS, 4xSCH_2), 2.0 (m, 8H, 4xC$H_2$$CH_2$OR) ; ^{13}CNMR 90.57MHz (CD_2Cl_2) (Refer to Table 31)

Hexakis(3-(1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosa-17-enyl)-4-thiabutyl)-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (39)



To a stirred solution of 2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylic acid (22.9mg, 43µmol) in methyl sulphoxide (20mL) at 68°C under argon, tetramethylammonium hydroxide (52mg, 287µmol, 1.1eq per. CO_2H) was added. To the resulting solution compound **17** (200mg, 302µmol,1.2eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3

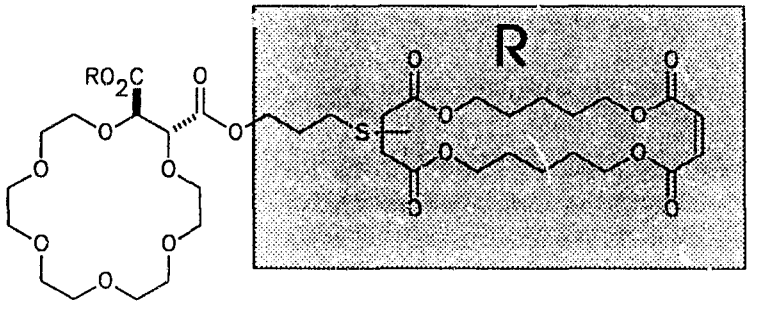
chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **12**, the product containing fractions were combined and the solvent removed to give **39** as a yellow oil (80mg, 21μmol, 49%): ^{1}HNMR 360MHz (δ, CD_2Cl_2): 6.3 (s, 12H, 6x*cis* C***H***=C***H***), 4.5-4.1 (m, 66H, 24x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 6x$CO_2C\boldsymbol{H}_2$ *crown* 6xC***H***O *methine*), 3.8-3.5 (br.m, 114H, 6x$C\boldsymbol{H}_2O$ *crown*, 48x$C\boldsymbol{H}_2O$, *macrocycle*, 6xSC***H***), 3.0-2.5 (m, 24H, 6x$C\boldsymbol{H}_2$CHS, 6xSC$\boldsymbol{H}_2$), 1.9 (m, 12H, 6x$C\boldsymbol{H}_2CH_2OR$) ; ^{13}CNMR 90.57MHz (CD_2Cl_2) (Refer to Table 31)

Table 31. ^{13}CNMR Data for compounds **37**, **38** and **39** (ppm)

Table 31 Assignment	**37**	**38**	**39**
C=O	171.1	171.5	171.5
	170.1	170.5	170.5
	165.0	165.4	165.5
			165.4
Crown	169.3	169.5	169.4
cis ***C***=***C***	129.7	130.1	130.2
	129.6	130.0	130.0
Crown ***C***HO	79.7	80.3	80.3

Table 31 Assignment	**37**	**38**	**39**
Macrocycle	71.0	70.9	70.9
$\mathbf{C}H_2O$	70.5	69.2	69.2
	70.3	69.1	69.1
	68.8	69.0	
	68.7		
Crown	Hidden	71.5	Hidden
$\mathbf{C}H_2O$		70.4	
Macrocycle	64.6	65.2	64.9
$CO_2\mathbf{C}H_2$	64.5	64.9	64.8
	64.2	64.8	64.5
		64.4	64.2
Crown	63.4	63.9	63.9
$CO_2\mathbf{C}H_2$			
$CH_2\mathbf{C}HS$	41.7	42.0	42.0
$\mathbf{C}H_2CHS$	36.4	36.8	36.8
$\mathbf{C}H_2$	28.1	28.5	28.5
	27.9	28.2	28.3

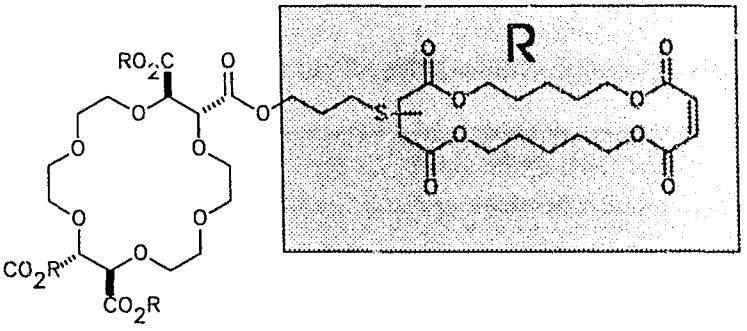
Bis(3-(1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosa-14-enyl)-4-thiabutyl)-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (40)



To a stirred solution of 2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylic acid (40mg, 114µmol) in methyl sulphoxide (20mL) at 70°C under argon, tetramethylammonium hydroxide (45mg, 249µmol, 1.1 eq per. CO_2H) was added. To this solution compound **23** (180mg, 416µmol, 1.4eq per. CO_2H) was added as a solution in methyl sulphoxide (2mL), and the mixture stirred for a further 12h. The resulting amber solution was evaporated to dryness, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **20**, the product containing fractions were combined and the solvent removed to give **40** as a yellow oil (80mg, 64µmol, 56%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 4H, 2x*trans* C***H***=C***H***), 4.4 (s, 2H, 2xC***H***C *methine*), 4.1 (m, 20H, 8x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 2x$CO_2C\boldsymbol{H}_2$ *crown*), 3.8 (m, 2H, 2xSC***H***), 3.6 (m, 20H 10x$C\boldsymbol{H}_2O$), 2.9-2.5 (m, 8H, 2x$C\boldsymbol{H}_2$CHS, 2xS$C\boldsymbol{H}_2$), 1.9 (m, 4H, 2x$C\boldsymbol{H}_2CH_2OR$), 1.8-1.4 (br.m, 24H, 12x$C\boldsymbol{H}_2$)

; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 32) ; Analaysis calculated for $C_{56}H_{84}O_{26}S_2$: C 54.36%; H 6.84%; S 5.18%; Analysis calculated with 1% DMSO : C 54.13%; H 6.84%; S 5.59%; Found C 53.94%; H 6.83%; S 5.94%

Tetrakis(3-(1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosa-14-enyl)-4-thiabutyl)-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (41)



To a stirred solution of 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxa-cyclooctadecane-2,3,11,12-tetracarboxylic acid (37mg, 84µmol) in methyl sulphoxide (20mL) at 70°C under argon, tetramethylammonium hydroxide (65mg, 359µmol, 1.1eq per. CO_2H) was added. To the resulting milky solution compound **23** (250mg, 439µmmol, 1.3eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **20**, the product containing

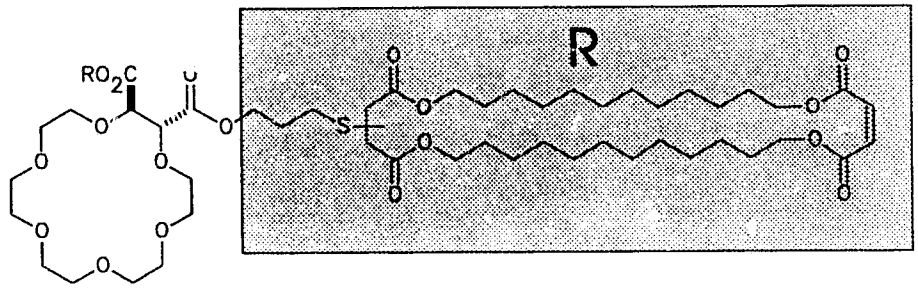
fractions were combined and the solvent removed to give **41** as a yellow oil (120mg, 54µmol, 64%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 8H, 4x*trans* C***H***=C***H***), 4.4-4.0 (m, 44H, 16x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 4x$CO_2C\boldsymbol{H}_2$ *crown*, 4xC***H***O *methine*), 3.8 (m, 4H, 4xSC***H***), 3.7-3.5 (m, 16H, 8x$C\boldsymbol{H}_2O$), 2.9-2.5 (m, 16H, 4x$C\boldsymbol{H}_2$CHS, 4x$SC\boldsymbol{H}_2$), 1.9 (m, 8H, 4x$C\boldsymbol{H}_2CH_2OR$), 1.8-1.5 (br.m, 48H, 24x$C\boldsymbol{H}_2$) ; ^{13}CNMR 62.89MHz ($CDCl_3$) (Refer to Table 32)

Table 32. ^{13}CNMR Data for compounds **40** and **41** (ppm)

Table 32 Assignment	**40**	**41**
C=O	171.0	171.2
	170.0	170.2
	164.8	165.9
	164.7	
Crown	169.2	169.3
trans ***C***=***C***	133.4	133.6
	133.3	133.5
Crown ***C***HO	79.6	80.1

Table 32 Assignment	**40**	**41**
Crown	71.1	71.8
$\mathbf{C}H_2O$	70.6	70.4
	70.5	
	70.3	
Macrocycle	64.9	65.1
$CO_2\mathbf{C}H_2$	64.5	64.7
Crown	63.4	63.6
$CO_2\mathbf{C}H_2$		
$CH_2\mathbf{C}HS$	42.4	42.6
$\mathbf{C}H_2CHS$	35.9	36.2
$\mathbf{C}H_2$	28.4	28.6
	28.3	28.5
	28.2	28.3
	28.1	28.2
	28.0	23.3
	27.0	23.2
	23.0	

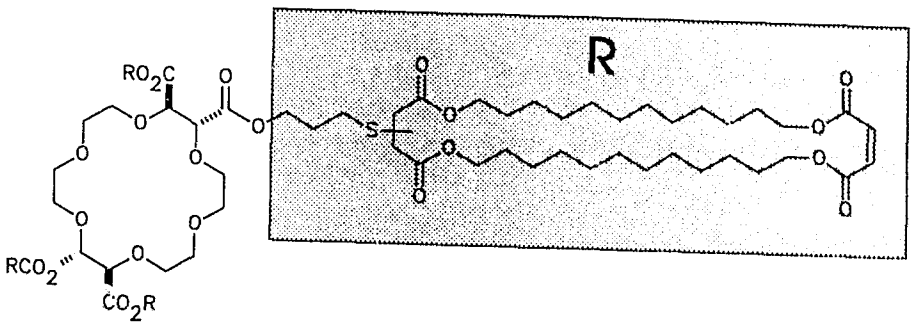
Bis(3-(1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatriconta-21-enyl)-4-thiabutyl)-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (42)



To a stirred solution of 2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylic acid (35mg, 99μmol) in methyl sulphoxide (20mL) at 80°C under argon, tetramethylammonium hydroxide (41mg, 224μmol, 1.1 eq per. CO_2H) was added. To this solution compound **28** (230mg, 300μmol, 1.5eq per. CO_2H) was added as a solution in methyl sulphoxide (2mL), and the mixture stirred for a further 12h. The resulting amber solution was evaporated to dryness, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **26**, the product containing fractions were combined and the solvent removed to give **42** as a yellow oil (100mg, 61μmol, 62%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 4H, 2x*trans* C***H***=C***H***), 4.4 (s, 2H, 2xC***H***O *methine*), 4.2-3.9 (m, 20H, 8x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 2x$CO_2C\boldsymbol{H}_2$ *crown*), 3.8 (m, 2H, 2xSC***H***), 3.6 (m, 20H 10x$C\boldsymbol{H}_2O$), 3.0-2.5 (m, 8H, 2x$C\boldsymbol{H}_2$CHS, 2xS$C\boldsymbol{H}_2$), 1.9 (m, 4H, 2x$C\boldsymbol{H}_2CH_2$OR), 1.7-1.1 (br.m, 80H, 40x$C\boldsymbol{H}_2$) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 33) ; Analysis

calculated for $C_{84}H_{140}O_{26}S_2$: C 61.89%; H 8.66%; S .93%; Analysis calculated with 1% DMSO : C 61.58%; H 8.66%; S 4.35%; Found C 61.61%; H 8.63%; S 4.84%

Tetrakis(3-(1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatricont 21-enyl)-4-thiabutyl)-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (43)



To a stirred solution of 2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclo-octadecane-2,3,11,12-tetracarboxylic acid (30mg, 68μmol) in methyl sulphoxide (20mL) at 80°C under argon, tetramethylammonium hydroxide (49mg, 273μmol, 1eq per. CO_2H) was added. To the resulting milky solution compound **28** (286mg, 373μmmol, 1.4eq per. CO_2H) was added as a solution (5mL, methyl sulphoxide) and the resulting mixture stirred for a further 12h. The resultant amber liquor was evaporated, dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 8-12 (1mL) eluted with 4:3 chloroform/methanol, later fractions contained **26**, the product containing

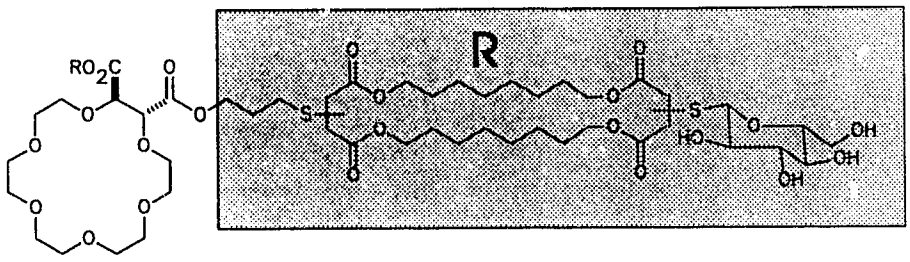
fractions were combined and the solvent removed to give **43** as a yellow oil (105mg, 35μmol, 51%): ^{1}HNMR 360MHz (δ, $CDCl_3$): 6.8 (s, 8H, 4x*trans* C***H***=C***H***), 4.3 (s, 4H, 4xC***H***O *methine*), 4.2-3.9 (m, 40H, 16x$CO_2C\boldsymbol{H}_2$ *macrocycle*, 4x$CO_2C\boldsymbol{H}_2$ *crown*), 3.8-3.4 (m, 20H, 8x$C\boldsymbol{H}_2O$, 4xSC***H***), 3.0-2.5 (m, 16H, 4x$C\boldsymbol{H}_2CHS$, 4x$SC\boldsymbol{H}_2$), 1.9 (m, 8H, 4x$C\boldsymbol{H}_2CH_2OR$), 1.8-1.5 (br.m, 160H, 80x$C\boldsymbol{H}_2$) ; ^{13}CNMR 90.57MHz ($CDCl_3$) (Refer to Table 33)

Table 33. ^{13}CNMR Data for compounds **42** and **43** (ppm)

Table 33 Assignment	**42**	**43**
C=O	171.7	171.3
	170.7	170.4
	165.3	165.0
Crown	169.7	169.3
trans ***C=C***	133.8	133.5
Crown ***C***HO	80.0	80.0
Crown	71.1	71.6
$\boldsymbol{C}H_2O$	70.8	70.4
	70.7	
	70.6	

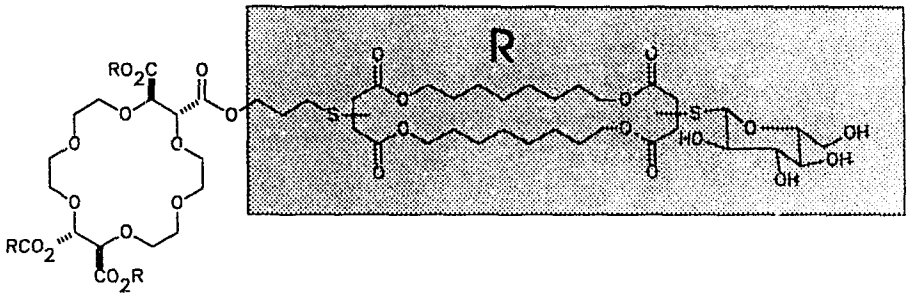
Table 33 Assignment	**42**	**43**
Macrocycle	65.7	65.4
$CO_2\mathbf{C}H_2$	65.4	65.1
Crown	63.9	63.5
$CO_2\mathbf{C}H_2$		
$CH_2\mathbf{C}HS$	42.0	41.6
$\mathbf{C}H_2CHS$	36.9	36.5
$\mathbf{C}H_2$	29.8	29.3
	29.7	29.0
	29.5	28.4
	28.9	28.2
	28.8	27.9
	28.6	26.0
	28.3	25.6
	26.4	
	26.1	
	26.0	

Bis[(3-(17and/or18-β-D-glucopyranosylthio)-(1,3,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (44)



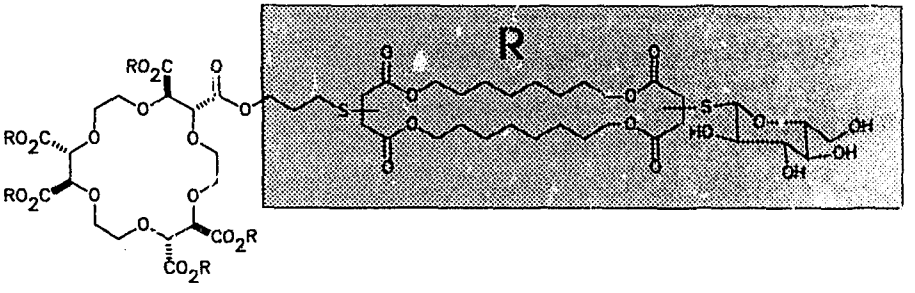
To a stirred solution of **29** (68.9mg, 48.6μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **44** as a clear oil (24.4mg, 13.6μmol, 28%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 34)

Tetrakis[(3-(17and/or18-β-D-glucopyranosylthio)-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (45)



To a stirred solution of **30** (65.0mg, 25.4μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol the product containing fractions were combined and the solvent removed to give **45** as a clear oil (28.5mg, 8.5μmol, 33%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 34)

Hexakis[(3-(17and/or18-β-D-glucopyranosylthio)-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (46)



To a stirred solution of **31** (64.0mg, 17.6μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **46** as a clear oil (25.9mg, 5.3μmol, 31%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 34)

Table 34. ^{13}CNMR Data for compounds **44**, **45** and **46** (ppm)

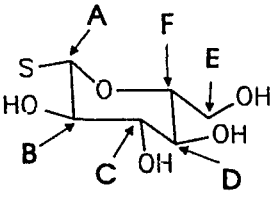
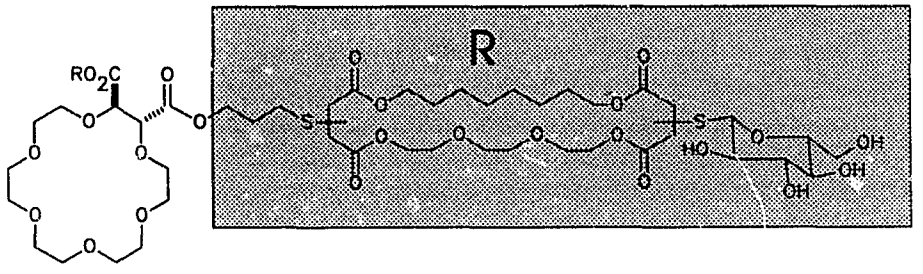
Table 34 Assignment	**44**	**45**	**46**
C=O	173.5	173.4	173.4
	173.3	173.3	173.3
	173.0	172.9	172.8
	172.2	172.2	172.2
	172.1	172.0	172.1
	171.9	171.9	171.9
Crown	171.3	171.0	170.8
Crown *C*HO	80.3	80.0	80.6
Glucose	86.4(A)	86.4(A)	86.5(A)
	85.8(A)	85.5(A)	85.8(A)
	82.1(F)	82.0(F)	82.1(F)
	82.0(F)	81.9(F)	82.0(F)
	79.6(B)	79.6(B)	79.6(B)
	79.5(B)	79.4(B)	79.5(B)
	74.3(D)	74.3(D)	74.3(D)
	62.8(E)	62.9(E)	62.9(E)

Table 34 Assignment	**44**	**45**	**46**
Glucose (C),	71.5	71.7	71.3
Crown $\mathbf{C}H_2O$	71.3	71.6	71.2
	70.9	71.2	
		70.9	
Macrocycle	66.7	66.8	66.8
$CO_2\mathbf{C}H_2$	66.5	66.5	66.6
	66.0	66.1	66.1
Crown $CO_2\mathbf{C}H_2$	65.3	65.3	65.6
$CH_2\mathbf{C}HS$	42.9	43.0	43.0
	42.8	42.8	42.8
	41.2	41.2	41.2
$\mathbf{C}H_2CHS$	38.8	38.8	38.0
	37.9	38.0	37.8
	37.7	37.8	

Table 34 Assignment	44	45	46
CH_2	30.3	30.4	30.4
	29.7	29.8	29.8
	29.5	29.0	29.5
	29.0	27.0	29.4
	26.9		29.1
			26.9

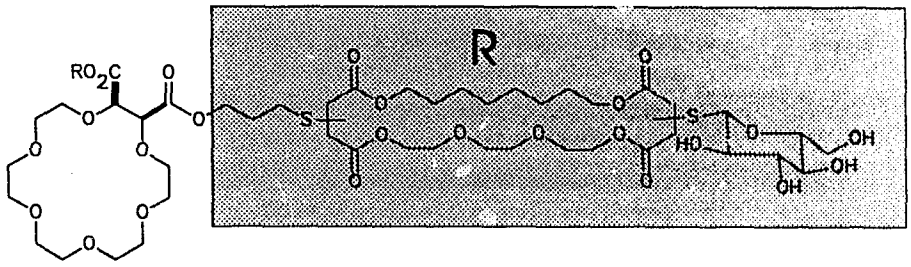
Bis[(3-and/or4-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (47)



To a stirred solution of **32** (69.0mg, 49.0μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was

removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **47** as a clear oil (14.6mg, 8.1μmol, 17%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 35)

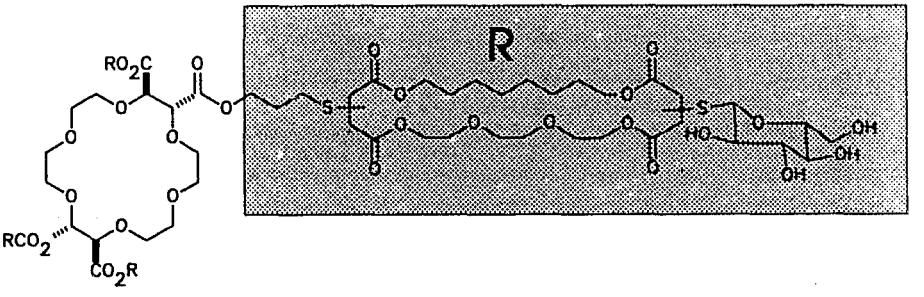
Bis[(3-and/or4-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3S-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (48)



To a stirred solution of **33** (60.0mg, 42.6μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product

containing fractions were combined and the solvent removed to give **48** as a clear oil (18.8mg, 10.4µmol, 24%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 35)

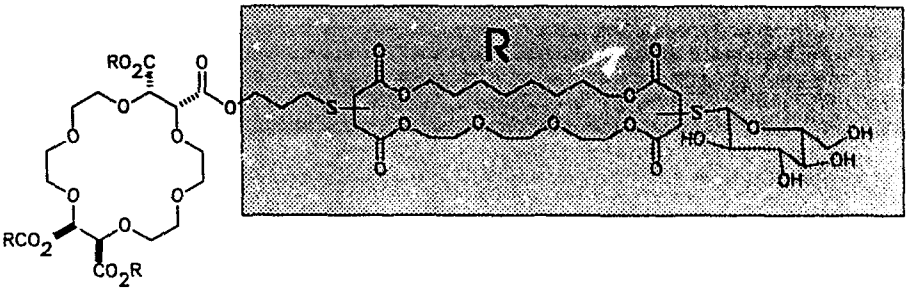
Tetrakis[(3-and/or4-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (49)



To a stirred solution of **34** (70.0mg, 27.3µmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200µmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197µmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **49** as a clear oil (11.2mg, 3.3µmol, 12%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz

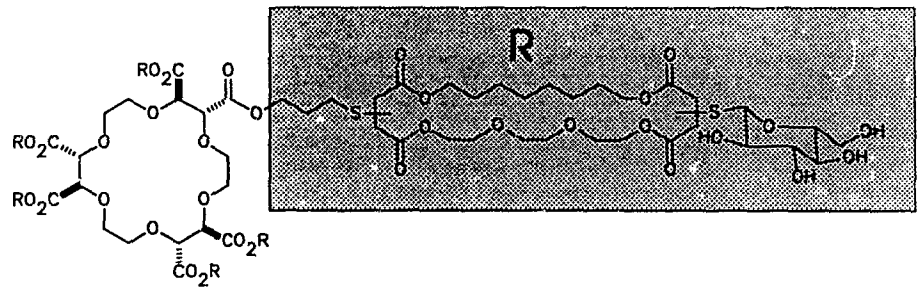
(CD_3OD)(Refer to Table 35)

Tetrakis[(3-and/or4-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,SR,11R,12S-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (50)



To a stirred solution of **35** (40.0mg, 15.7μmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 3eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **50** as a clear oil (10.3mg, 3.0μmol, 19%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 35)

Hexakis[(3-and/or4-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (51)



To a stirred solution of **36** (50.3mg, 13.7μmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2.5eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **51** as a clear oil (32.0mg, 6.5μmol, 47%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 35)

Table 35. ^{13}CNMR Data for compounds **47, 48, 49, 50** and **51** (ppm)

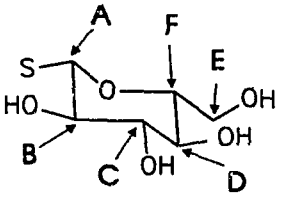
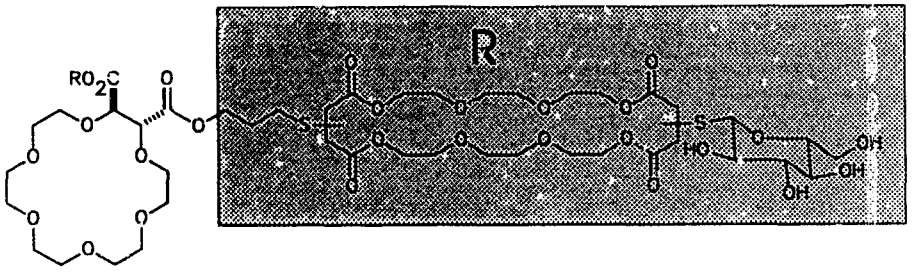
Table 35 Assignment	**47**	**48**	**49**	**50**	**51**
C=O	173.4	173.5	173.5	173.5	173.5
	173.3	173.3	173.4	173.4	173.4
	173.0	173.1	173.0	172.1	172.9
	172.2	172.2	172.2	172.2	172.8
	172.0	172.1	172.0		172.2
					172.1
					172.0
					171.9
Crown	171.3	171.0	171.1	170.6	170.8
Crown *C*HO	80.4	81.6	79.8	Hidden	80.5
Glucose	86.4(A)	86.5(A)	86.5(A)	86.5(A)	86.5(A)
	85.8(A)	85.8(A)	85.8(A)	85.8(A)	85.8(A)
	82.0(F)	81.9(F)	82.0(F)	81.9(F)	81.9(F)
	81.9(F)	81.8(F)			81.8(F)
	79.6(B)	79.6(B)	79.6(B)	79.6(B)	79.6(B)
	79.5(B)	79.5(B)	79.4(B)		79.5(B)
	74.3(D)	74.3(D)	74.3(D)	74.3(D)	74.3(D)
	62.8(E)	62.9(E)	62.8(E)	62.8(E)	62.9(E)

Table 35 Assignment	47	48	49	50	51
Glucose(C),	71.6	71.9	71.7	71.6	71.6
Macrocycle CH_2O,	71.3	71.8	71.4	71.3	71.3
Crown CH_2O	70.9	71.6	70.4	70.0	71.2
	70.0	71.5	70.0		70.0
		71.3			
		71.2			
		70.0			
Macrocycle	66.7	66.7	66.8	64.7	66.6
CO_2CH_2	66.4	66.4	66.5	66.4	66.5
	65.9	66.0	66.0	66.0	65.9
	65.7	65.8	65.8		65.8
		65.2			
Crown CO_2CH_2	65.1	64.8	65.2	65.2	65.2
CH_2CHS	42.9	43.0	43.1	43.1	42.8
	42.7	42.8	41.4	41.3	42.7
	41.2	41.2	41.1	41.1	41.2
CH_2CHS	37.8	38.6	38.6	38.6	38.5
	37.6	37.7	37.9	37.8	37.5
	37.5	37.6	37.6	37.6	37.4

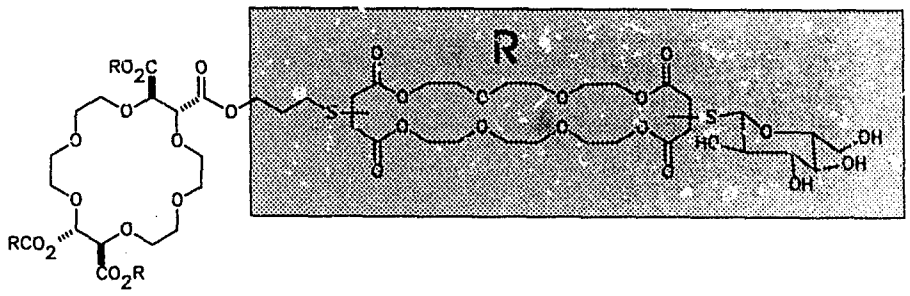
Table 35 Assignment	**47**	**48**	**49**	**50**	**51**
CH_2	30.1	30.1	30.8	29.9	29.9
	29.5	29.9	29.8	29.6	29.6
	28.9	29.6	29.6	29.1	29.1
	26.8	29.0	29.1	26.7	26.7
	26.6	26.7	26.7		

Bis[(3-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (52)



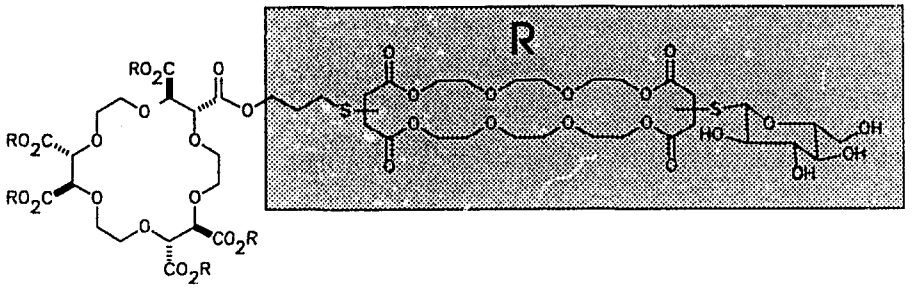
To a stirred solution of **37** (71.0mg, 50.0μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **52** as a clear oil (21.7mg, 12.0μmol, 24%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 36)

Tetrakis[(3-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (53)



To a stirred solution of **38** (56.1mg, 21.9μmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **53** as a clear oil (19.5mg, 5.3μmol, 26%) ; ^{1}HNMR 360MHz (CD_3OD)[51] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 36)

Hexakis[(3-(17and/or18-β-D-glucopyranosylthio)-(1,6,9,12,15,20,23,24-octaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (54)



To a stirred solution of **39** (17.0mg, 4.5μmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 7eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **54** as a clear oil (4.5mg, 915nmol, 20%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 36)

Table 36. ^{13}CNMR Data for compounds **52**, **53** and **54** (ppm)

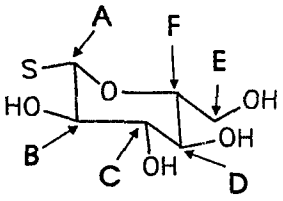
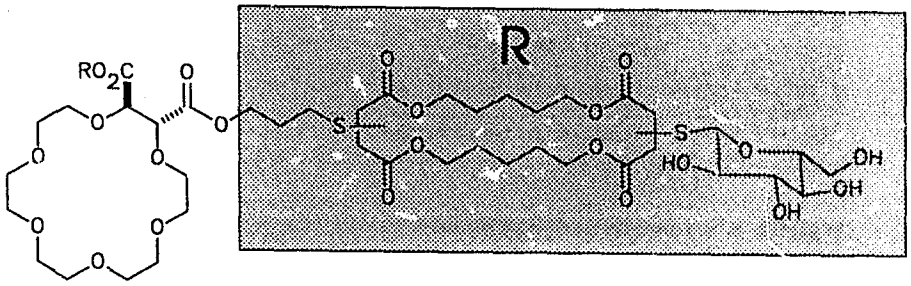
Table 36 Assignment	**52**	**53**	**54**
C=O	173.4	173.4	173.4
	173.3	173.2	173.2
	173.1	173.1	172.2
	172.2	173.0	172.1
	171.9	172.2	172.0
	172.0	172.1	
		172.0	
		171.9	
Crown	171.7	171.1	170.9
Crown CHO	79.9	79.9	80.5
Glucose	86.4(A)	86.4(A)	86.4(A)
	85.6(A)	85.6(A)	85.6(A)
	82.0(F)	82.0(F)	82.0(F)
	81.9(F)	81.9(F)	81.9(F)
	79.6(B)	79.6(B)	79.6(B)
	79.5(B)	79.5(B)	79.5(B)
	74.3(D)	74.3(D)	74.3(D)
	62.9(E)	62.8(E)	62.8(E)

Table 36 Assignment	**52**	**53**	**54**
Glucose(C),	71.3	71.3	71.3
Macrocycle CH_2O,	71.2	71.2	71.2
Crown CH_2O	70.9	70.5	
	70.4	70.3	
Macrocycle CH_2O	71.6	71.6	71.6
	69.9	70.0	70.0
Macrocycle	66.1	66.0	66.1
CO_2CH_2	65.8	65.8	66.0
Crown CO_2CH_2	65.3	65.3	65.8
CH_2CHS	42.9	42.9	42.9
	42.5	42.6	42.6
	41.1	41.1	41.1
CH_2CHS	39.5	39.5	38.6
	38.6	38.6	37.7
	37.7	37.7	37.6
	37.5	37.5	

Table 36 Assignment	52	53	54
CH_2	29.4	29.5	29.8
	28.9	29.4	29.1
		29.0	

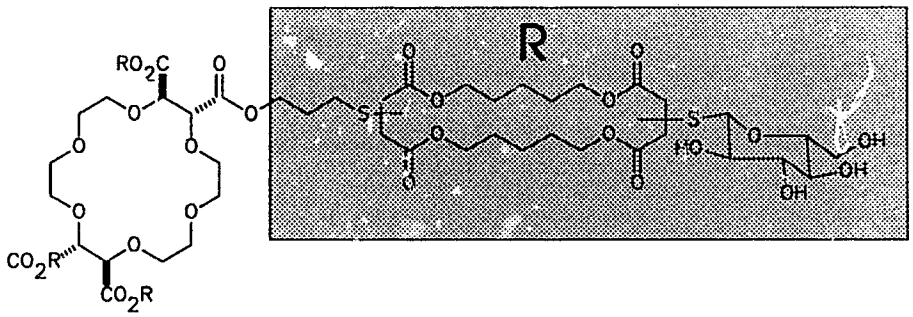
Bis[(3-(14and/or15-β-D-glucopyranosylthio)-(1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosyl)-4-thiabutyl)]-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (55)



To a stirred solution of **40** (26.7mg, 21.5μmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 2eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing

fractions were combined and the solvent removed to give **55** as a clear oil (5.6mg, 3.4μmol, 16%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 37)

Tetrakis[(3-(14and/or15-β-D-glucopyranosylthio)-(1,6,12,17-tetraoxa-2,5,13,16-tetraoxocyclodicosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (56)



To a stirred solution of **41** (73.9mg, 33.4μmol) in 50:50 2-propanol/ tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 1.5eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **56** as a clear oil (4.0mg, 1.3μmol, 4%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz

(CD_3OD)(Refer to Table 37)

Table 37. ^{13}CNMR Data for compounds **55** and **56** (ppm)

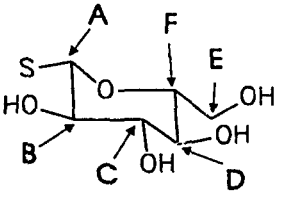
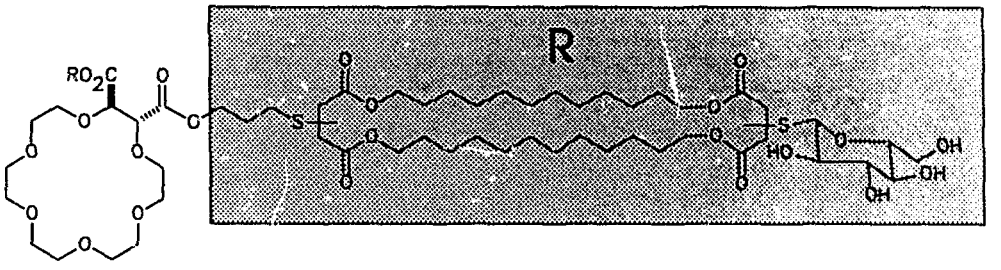
Table 37 Assignment	**55**	**56**
C=O	173.0	173.5
	172.0	173.4
		172.2
		172.1
		172.0
Crown	171.1	171.2
Crown ***C***HO	80.7	80.1
Glucose 	86.4(A)	86.5(A)
	85.8(A)	85.7(A)
	82.1(F)	82.1(F)
	82.0(F)	82.0(F)
	79.6(B)	79.8(B)
	79.5(B)	79.7(B)
	74.3(D)	74.5(D)
	62.9(E)	62.9(E)

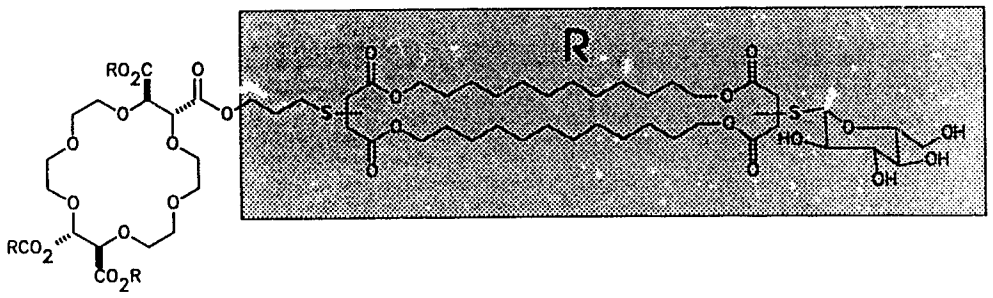
Table 37 Assignment	**55**	**56**
Glucose (C),	71.8	71.4
Crown $\boldsymbol{C}H_2O$	71.7	71.3
	71.6	70.8
		70.4
Macrocycle	66.5	66.3
$CO_2\boldsymbol{C}H_2$	66.0	66.2
		65.8
Crown $CO_2\boldsymbol{C}H_2$	65.0	65.4
$CH_2\boldsymbol{C}HS$	43.0	43.0
	42.9	42.8
	41.1	41.2
$\boldsymbol{C}H_2CHS$	38.7	38.8
	37.7	38.0
	37.5	37.8
$\boldsymbol{C}H_2$	29.5	30.4
	29.2	29.8
	27.7	29.0
	23.2	27.0

Bis[(3-(21and/or22-β-D-glucopyranosylthio)-(1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatricontyl)-4-thiabutyl)]-2R,3R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3-dicarboxylate (57)



To a stirred solution of **42** (27.0mg, 16.6μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (19mg, 200μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (50mg, 197μmol, 6eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **57** as a clear oil (6.7mg, 3.3μmol, 20%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 38)

Tetrakis[(3-(21and/or22-β-D-glucopyranosylthio)-(1,6,19,24-tetraoxa-2,5,20,23-tetraoxocyclohexatricontyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (58)



To a stirred solution of **43** (55.0mg, 18.3μmol) in 50:50 2-propanol/tetrahydofuran (20mL), at 50°C under nitrogen, methane sulphonic acid (11mg, 111μmol) as a 2-propanol solution was added. To this solution 1-thio-β-D-glucose sodium salt dihydrate (28mg, 110μmol, 1.5eq per alkene, 1eq per acid) was added (pH 6), 2,2,6,6-tetramethylpiperidine (5 drops) was added (pH 8), and the cloudy solution stirred a further 12h. The solvent was removed, and the product dissolved in 4:3 chloroform/methanol (2mL), filtered and added to a prewashed LH-20 (4x20cm) column. The product was collected in fractions 7-13 (1mL) eluted with 4:3 chloroform/methanol, the product containing fractions were combined and the solvent removed to give **58** as a colourless solid (10.4mg, 2.8μmol, 15%) ; ^{1}HNMR 360MHz (CD_3OD)[54] ; ^{13}CNMR 90.57MHz (CD_3OD)(Refer to Table 38)

Table 38. ^{13}CNMR Data for compounds **57** and **58** (ppm)

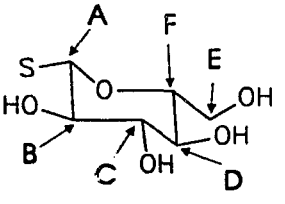
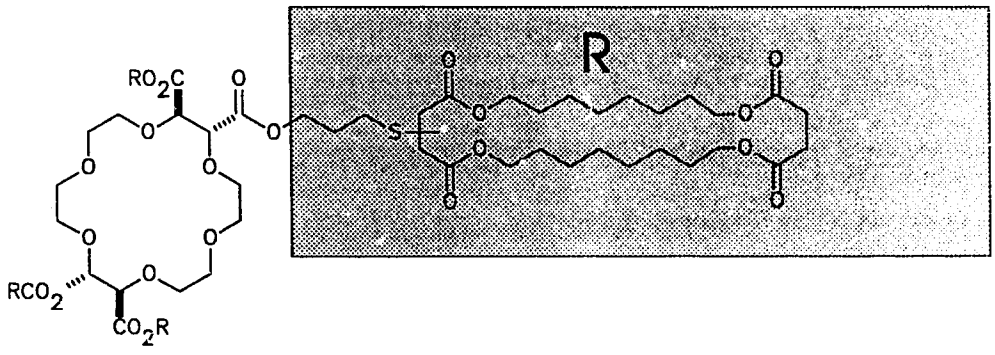
Table 38 Assignment	**57**	**58**
***C*=O**	173.4	173.4
	173.3	172.9
	172.9	172.1
	172.2	171.9
	172.0	
	171.9	
Crown	171.3	171.0
Crown ***C*HO**	80.3	79.8
Glucose 	86.4(A)	86.5(A)
	85.8(A)	85.8(A)
	82.0(F)	82.1(F)
	81.9(F)	
	79.6(B)	79.7(B)
	79.5(B)	
	74.3(D)	74.3(D)
	62.9(E)	62.9(E)
	62.8(E)	

Table 38 Assignment	57	58
Glucose(C),	71.4	71.4
Crown CH_2O	71.3	70.6
	71.2	
	70.9	
Macrocycle	66.8	66.6
CO_2CH_2	66.6	66.1
	66.5	
	66.1	
Crown CO_2CH_2	65.2	65.6
CH_2CHS	43.0	43.0
	42.8	42.8
	41.2	41.3
CH_2CHS	38.7	38.8
	37.9	37.7
	37.6	

Table 38 Assignment	57	58
CH_2	30.8	30.8
	30.4	30.5
	29.8	29.8
	328.9	29.0
	27.1	27.1

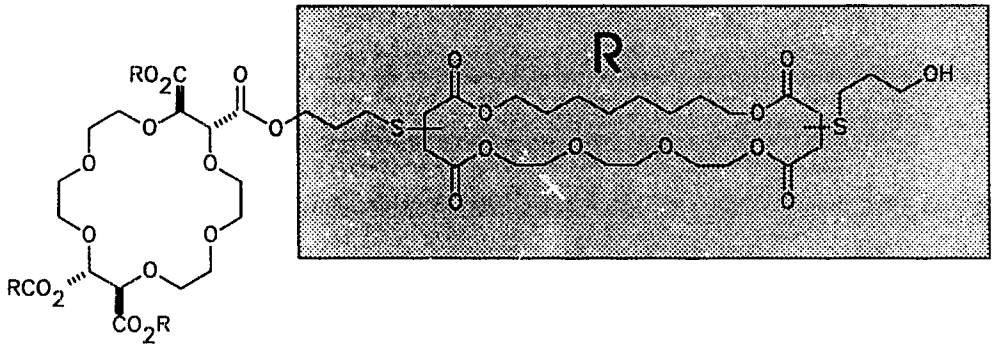
Tetrakis[(3-(17and/or18-(4-hydroxy-1-thiabutyl))-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (59)



Compound **30** (70.0mg, 27.5µmol) and 3-mercapto-1-propanol (34.2mg, 372µmol, 3eq per alkene) as a 2-propanol solution were added to 2-propanol (20mL), piperidine (5drops) was added and the mixture refluxed for 1h. The solvent was removed and the product dissolved in 4:3 chloroform/methanol (2ml) and added to a LH 20 (4x20cm) column. The product was collected in fractions 8-10 (1mL) eluted with 4:3 chloroform/methanol, product containing

fractions were combined and the solvent removed to give **59** as a yellow oil (36.6mg, 12.6µmol, 46%): ^{1}HNMR 360MHz ($CDCl_3$)[54] ; ^{13}CNMR 62.89MHz (δ, $CDCl_3$): 171.5 (*C*=O),171.2 (*C*=O), 170.3 (*C*=O), 169.2 (*C*=O *crown*), 80.0 (*C*HO *crown methine*), 71.5 (CH_2O *crown*), 70.3 (CH_2O *crown*), 65.5 (CO_2CH_2 *macrocycle*), 65.0 (CO_2CH_2 *macrocycle*), 63.5 (CO_2CH_2 *crown*), 61.2 (CH_2OH), 41.9 (*C*HS), 41.7 (*C*HS), 36.7 (CH_2S *macrocycle*), 31.9 (CH_2S *link* and $SCH_2CH_2CH_2OH$), 29.1 (CH_2), 28.5 (CH_2), 27.9 (CH_2), 25.8 (CH_2)

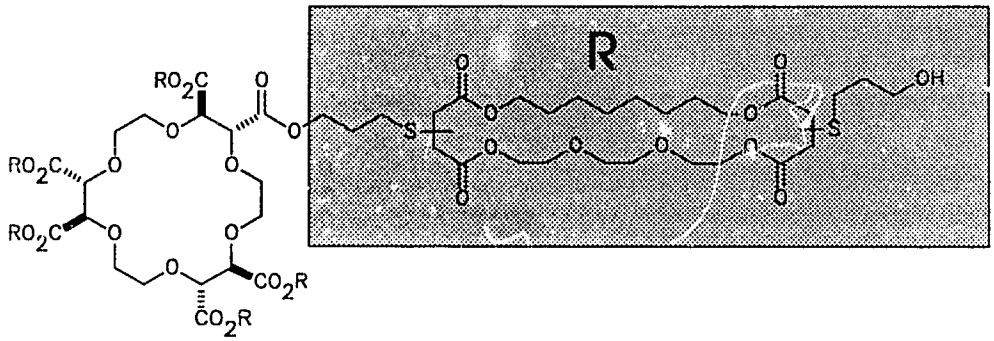
Tetrakis[(3-and/or4-(17and/or18-(4-hydroxy-1-thiabutyl))-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (60)



Compound **34** (71.1, 27.8µmol) and 3-mercapto-1-propanol (32.7mg, 355µmol, 3eq per alkene) as a 2-propanol solution were added to tetrahydrofuran (20mL) at 50°C, 2,2,6,6-Tetramethylpiperidine (5drops) was added and the mixture stirred a further 3h. The solvent was removed and the product dissolved in 4:3 chloroform/methanol (2ml) and added to a LH 20 (4x20cm) column. The product was collected in fractions 8-10 (1mL) eluted

with 4:3 chloroform/methanol, product containing fractions were combined and the solvent removed to give **60** as a yellow oil (34.8mg, 11.8μmol, 42%) : ^{1}HNMR 360MHz ($CDCl_3$)[54] ; ^{13}CNMR 90.57MHz (δ, $CDCl_3$): 171.5 (***C***=O),171.4 (***C***=O), 171.3 (***C***=O), 171.2 (***C***=O), 170.3 (***C***=O), 170.2 (***C***=O), 169.1 (***C***=O *crown*), 79.9 (***C***HO *crown methine*), 71.3 ($C\mathbf{H_2}$O *crown*), 70.2 ($C\mathbf{H_2}$O *crown*), 70.5 ($C\mathbf{H_2}$O *macrocycle*), 68.9 ($C\mathbf{H_2}$O *macrocycle*), 65.3 ($CO_2C\mathbf{H_2}$ *macrocycle*), 64.9 ($CO_2C\mathbf{H_2}$ *macrocycle*), 64.3 ($CO_2C\mathbf{H_2}$ *macrocycle*), 63.9 ($CO_2C\mathbf{H_2}$ *macrocycle*), 63.5 ($CO_2C\mathbf{H_2}$ *crown*), 60.9 ($C\mathbf{H_2}$OH), 41.7 (***C***HS), 41.6 (***C***HS *macrocycle*), 41.4 (***C***HS *macrocycle*), 36.5 ($C\mathbf{H_2}$S *macrocycle*). 36.4 ($C\mathbf{H_2}$S *macrocycle*), 36.3 ($C\mathbf{H_2}$S *macrocycle*), 31.8 ($C\mathbf{H_2}$S *link* and S$C\mathbf{H_2}CH_2CH_2$OH), 31.7 ($C\mathbf{H_2}$S *link* and S$C\mathbf{H_2}CH_2CH_2$OH), 28.7 ($C\mathbf{H_2}$), 28.6 ($C\mathbf{H_2}$), 28.3 ($C\mathbf{H_2}$), 28.0 ($C\mathbf{H_2}$), 27.9 ($C\mathbf{H_2}$), 27.7 ($C\mathbf{H_2}$), 25.4 ($C\mathbf{H_2}$), 25.3 ($C\mathbf{H_2}$)

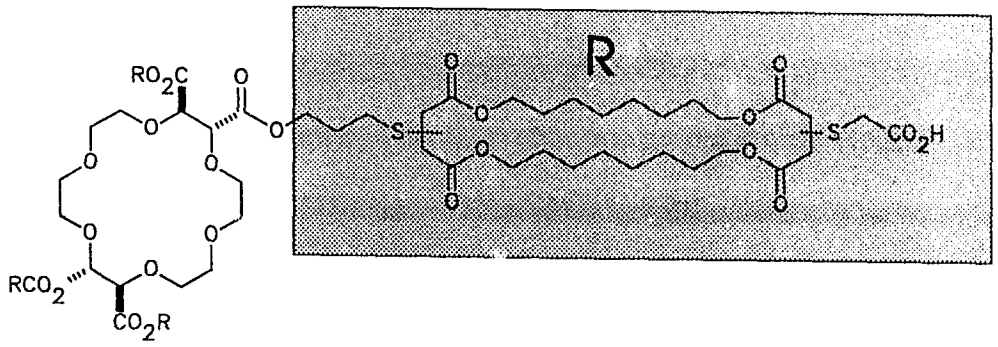
Hexakis[(3-and/or4-(17and/or18-(4-hydroxy-1-thiabuyl))-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (61)



Compound **36** (90.0mg, 24.3μmol) and 3-mercapto-1-propanol (32.7mg, 355μmol, 2eq per alkene) as a 2-propanol solution were added to tetrahydrofuran (20mL) at 50°C, 2,2,6,6-Tetramethylpiperidine (5drops) was added and the mixture stirred a further 3h. The solvent was removed and the product dissolved in 4:3 chloroform/methanol (2ml) and added to a LH 20 (4x20cm) column. The product was collected in fractions 8-10 (1mL) eluted with 4:3 chloroform/methanol, product containing fractions were combined and the solvent removed to give **61** as a yellow oil (33.8mg, 7.9μmol, 33%) : ^{1}HNMR 360MHz ($CDCl_3$)[54] ; ^{13}CNMR 90.57MHz (δ, $CDCl_3$): 171.6 (***C***=O),171.5 (***C***=O), 171.3 (***C***=O), 171.2 (***C***=O), 170.4 (***C***=O), 170.3 (***C***=O), 169.1 (***C***=O *crown*), 80.0 (***C***HO *crown methine*), 70.5 ($\mathbf{C}H_2O$ *macrocycle*), 68.9 ($\mathbf{C}H_2O$ *macrocycle*), 65.4 ($CO_2\mathbf{C}H_2$ *macrocycle*), 64.9 ($CO_2\mathbf{C}H_2$ *macrocycle*), 64.4 ($CO_2\mathbf{C}H_2$ *macrocycle*), 63.9 ($CO_2\mathbf{C}H_2$ *macrocycle*), 63.6 ($CO_2\mathbf{C}H_2$ *crown*), 61.0 ($\mathbf{C}H_2OH$), 41.8 (***C***HS),

41.7 (*C*HS *macrocycle*), 36.6 (*C*H_2S *macrocycle*), 36.5 (*C*H_2S *macrocycle*), 36.4 (*C*H_2S *macrocycle*), 31.9 (*C*H_2S *link* and S*C*H_2CH_2CH_2OH), 31.8 (*C*H_2S *link* and SCH_2CH_2CH_2OH), 28.8 (*C*H_2), 28.4 (*C*H_2), 28.1 (*C*H_2), 28.0 (*C*H_2), 27.8 (*C*H_2), 25.5 (*C*H_2), 25.4 (*C*H_2), 25.3 (*C*H_2)

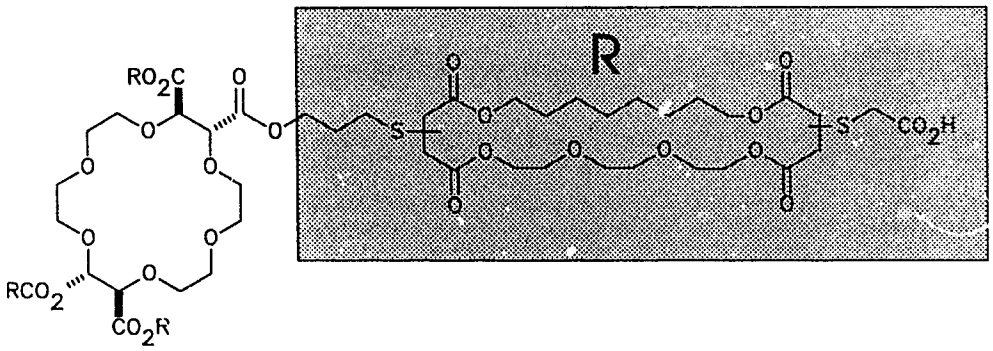
Tetrakis[(3-(17and/or18-(1-thio-2-carboxylethyl)-(1,6,15,20-tetraoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (62)



Compound **30** (68.0mg, 26.7μmol) and mercaptoacetic acid (32.0mg, 347μmol, 3eq per alkene) as a 2-propanol solution were added to tetrahydrofuran (20mL) at 50°C, 2,2,6,6-Tetramethylpiperidine (5drops, pH 8) was added and the mixture stirred a further 3h. The solvent was removed and the product dissolved in 4:3 chloroform/methanol (2ml) and added to a Dowex 50x8-100 ion exchange resin (1x5cm) column, which had been activated with 2M sulphuric acid and washed to neutral with water, then methanol (50mL), and 4:3 chloroform/methanol (50mL). The acidic fractions were combined and concentrated (~1mL) and added to a LH 20 (4x20cm) column. The product was

collected in fractions 8-10 (1mL) eluted with 4:3 chloroform/methanol, product containing fractions were combined and the solvent removed to give **62** as a yellow oil (32.0mg, 10.9μmol, 41%): ^{1}HNMR 360MHz ($CDCl_3$)[54] ; ^{13}CNMR 90.57MHz (δ, $CDCl_3$): 173.3 (***C***=O ***C***O_2H), 171.2 (***C***=O), 170.8 (***C***=O), 170.4 (***C***=O), 170.3 (***C***=O), 170.0 (***C***=O), 169.2 (***C***=O *crown*), 79.9 (***C***HO *crown methine*), 71.3 (***C***H_2O *crown*), 70.2 (***C***H_2O *crown*), 65.6 (CO_2***C***H_2 *macrocycle*), 65.5 (CO_2***C***H_2 *macrocycle*), 65.0 (CO_2***C***H_2 *macrocycle*), 63.5 (CO_2***C***H_2 *crown*), 41.8 (***C***HS), 41.6 (***C***HS), 36 5 (***C***H_2CHS), 36.2 (***C***H_2CHS), 33.0 (***C***H_2CO_2H), 29.0 (***C***H_2), 28.4 (***C***H_2), 28.1 (***C***H_2), 27.9 (***C***H_2), 25.7 (***C***H_2), 25.6 (***C***H_2)

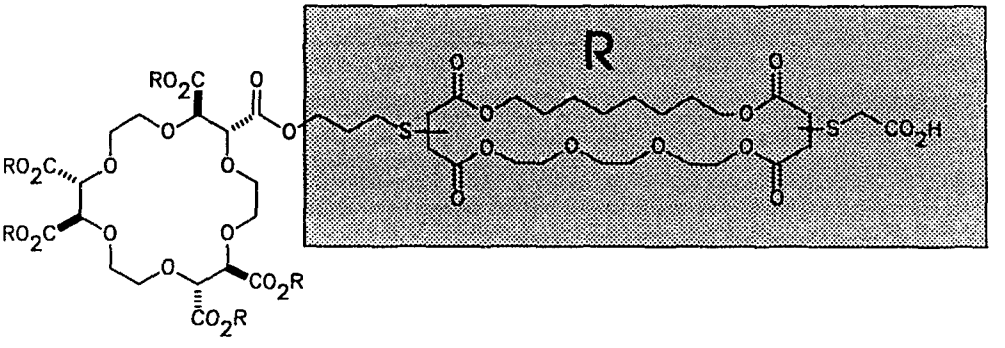
Tetrakis[(3-and/or4-(17and/or18-(1-thio-2-carboxylethyl))-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)]-2R,3R,11R,12R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,11,12-tetracarboxylate (63)



Compound **34** (80.9mg, 31.6μmol) and mercaptoacetic acid (32.0mg, 347μmol, 3eq per alkene) as a 2-propanol solution were added to tetrahydrofuran (20mL) at 50°C, 2,2,6,6-Tetramethylpiperidine (5drops, pH 8) was added and the mixture stirred a further 3h. The solvent was removed and

the product dissolved in 4:3 chloroform/methanol (2ml) and added to a Dowex 50x8-100 ion exchange resin (1x5cm) column, which had been activated with 2M sulphuric acid and washed to neutral with water, then methanol (50mL), and 4:3 chloroform/methanol (50mL). The acidic fractions were combined and concentrated (~1mL) and added to a LH 20 (4x20cm) column. The product was collected in fractions 8-10 (1mL) eluted with 4:3 chloroform/methanol, product containing fractions were combined and the solvent removed to give **63** as a yellow oil (19.2mg, 6.5μmol, 21%) : ^{1}HNMR 360MHz ($CDCl_3$)[54] ; ^{13}CNMR 90.57MHz (δ, $CDCl_3$): 173.3 (***C***=O), 171.0 (***C***=O), 170.5 (***C***=O), 169.2 (***C***=O *crown*), 79.0 (***C***HO *crown methine*), 71.3 ($\mathbf{C}H_2O$ *crown*), 70.2 ($\mathbf{C}H_2O$ *crown*), 70.5 ($\mathbf{C}H_2O$ *macrocycle*), 68.8 ($\mathbf{C}H_2O$ *macrocycle*), 65.5 ($CO_2\mathbf{C}H_2$ *macrocycle*), 65.4 ($CO_2\mathbf{C}H_2$ *macrocycle*), 64.9 ($CO_2\mathbf{C}H_2$ *macrocycle*), 64.6 ($CO_2\mathbf{C}H_2$ *macrocycle*), 64.0 ($CO_2\mathbf{C}H_2$ *macrocycle*), 63.7 ($CO_2\mathbf{C}H_2$ *crown*), 42.2 (***C***HS), 41.6 (***C***HS), 41.5 ($\mathbf{C}H_2S$), 36.3 ($\mathbf{C}H_2CHS$), 35.9 ($\mathbf{C}H_2CHS$), 33.7 ($\mathbf{C}H_2CO_2H$), 33.0 ($\mathbf{C}H_2CO_2H$) 28.6 ($\mathbf{C}H_2$), 28.5 ($\mathbf{C}H_2$), 28.3 ($\mathbf{C}H_2$), 28.0 ($\mathbf{C}H_2$), 27.9 ($\mathbf{C}H_2$), 25.4 ($\mathbf{C}H_2$), 25.3 ($\mathbf{C}H_2$)

Hexakis[(3-and/or4-(17and/or18-(1-thio-2-carboxylethyl))-(1,6,9,12,20-hexaoxa-2,5,16,19-tetraoxocyclooctacosyl)-4-thiabutyl)-2R,3R,8R,9R,14R,15R-1,4,7,10,13,16-hexaoxacyclooctadecane-2,3,8,9,14,15-hexacarboxylate (64)



Compound **36** (48.0mg, 12.9μmol) and mercaptoacetic acid (21.3mg, 321μmol, 3eq per alkene) as a 2-propanol solution were added to tetrahydrofuran (20mL) at 50°C, 2,2,6,6-Tetramethylpiperidine (5drops, pH 8) was added and the mixture stirred a further 3h. The solvent was removed and the product dissolved in 4:3 chloroform/methanol (2ml) and added to a Dowex 50x8-100 ion exchange resin (1x5cm) column, which had been activated with 2M sulphuric acid and washed to neutral with water, then methanol (50mL), and 4:3 chloroform/methanol (50mL). The acidic fractions were combined and concentrated (~1mL) and added to a LH 20 (4x20cm) column. The product was collected in fractions 8-10 (1mL) eluted with 4:3 chloroform/methanol, product containing fractions were combined and the solvent removed to give **64** as a yellow oil (12.1mg, 2.8μmol, 22%) : ^{1}HNMR 360MHz ($CDCl_3$)[54] ; ^{13}CNMR 90.57MHz (δ, $CDCl_3$): 171.9 (***C***=O), 171.4 (***C***=O), 171.2 (***C***=O), 170.4 (***C***=O),

169.0 (***C***=O), 169.0 (***C***=O *crown*), 79.9 (***C***HO *crown methine*), 70.4 (***C***H_2O *macrocycle*), 70.3 (***C***H_2O *macrocycle*), 69.3 (***C***H_2O *macrocycle*), 68.9 (***C***H_2O *macrocycle*), 65.6 (CO_2***C***H_2 *macrocycle*), 65.4 (CO_2***C***H_2 *macrocycle*), 64.9 (CO_2***C***H_2 *macrocycle*), 64.7 (CO_2***C***H_2 *macrocycle*), 64.4 (CO_2***C***H_2 *macrocycle*), 64.0 (CO_2***C***H_2 *macrocycle*), 63.6 (CO_2***C***H_2 *crown*), 42.3 (***C***HS), 41.7 (***C***HS), 36.3 (***C***H_2CHS), 35.9 (***C***H_2CHS), 33.6 (***C***H_2CO_2H), 32.9 (***C***H_2CO_2H) 28.3 (***C***H_2), 28.1 (***C***H_2), 27.9 (***C***H_2), 25.6 (***C***H_2), 25.3 (***C***H_2)

APPENDIX I

This experimental procedure is taken with minor changes from the Masters Thesis of K. C. Kaye

General Instruments

The titration system was a Metrohm 655 Dosimat buret and titration cell, 614 Impulsomat automatic titrator, and 632 pH-meter. The buret was linked to an HP-85 microcomputer for data acquisition. Vesicle preparation required use of a Heat Systems W385 Ultrasonic sonicator, located in the University of Victoria's Biochemistry and Microbiology Departments. Size distribution analysis of vesicles was performed using a Philips EM360 Transmission Electron Microscope, located in the Biology Department.

Vesicle Preparation

Egg phosphatidylcholine and egg phosphatidic acid (egg PC and PA) were purchased from Avanti Polar Lipids, Inc., Pelham, Alabama. Cholesterol was purchased from Sigma/Aldrich. Anhydrous diethyl ether and HPLC grade chloroform were purchased from BDH; methanol, tetrahydrofuran was distilled from potassium, choline hydroxide (20% in water), D-mannitol, and Bis-Tris (2,2-bis(hydroxymethyl)-2,2',2"-nitrilotriethanol) from Sigma/Aldrich; sulphuric acid (ultrapure) from Fluka; and PD-10 Sephadex G-25M columns from Pharmacia. Only D^3 (deionized, double distilled) water was used.

Stock Solutions

Choline Sulphate - Choline hydroxide (250 mL of 20% solution) was titrated

to pH 6.5 with concentrated sulphuric acid. Activated charcoal was added, and the solution stirred for 10 minutes; the charcoal was removed by filtration through Celite, and the solution concentrated by rotary evaporation. One half liter of 100% ethanol was added and the tarry residue was dissolved with heating. Anhydrous ether (200 mL) was added and the solution cooled to -10 °C for 16 hours; the crystalline precipitate was removed by filtration. The mother liquor was returned to the freezer for overnight to yield a final crop of precipitate. The combined batches of choline sulphate precipitate were dried for 24 hours by vacuum.

Internal Buffer Solution - 0.20 M bis-tris, and 0.054 M D-mannitol; the pH was adjusted to 6.60 using 0.45 M H_2SO_4. Internal buffer was made in 500 mL batches.

External Solution - 0.110 M choline sulphate, and 0.093 M D-mannitol; made in one liter batches, portioned into 200 mL glass bottles and kept refrigerated as much as possible. Filtration through Millipore GS 0.22 µm filters extended the life of external solution.

Choline Hydroxide Titrant - one liter of 4.75 mM choline hydroxide, and 0.35 M D-mannitol, dissolved in D^3 water that was boiled for 30 minutes to release carbon dioxide. This solution was made in a nitrogen filled glove bag to avoid carbon dioxide contamination. The base titer was established by titration versus standard potassium hydrogen phthalate.

Egg Lecithin Vesicle Preparation

The egg lecithin vesicles were made by reverse evaporation, followed by sizing filtration and size exclusion chromatography. Each batch generated vesicles for approximately 16 pH-stat experiments. The following prescriptive procedure yields the most reproducible and reliable batches of vesicles for pH-stat work; that is, most of the entrapped buffer is within large unilamellar vesicles.

The vesicle bilayer composition is an 8:1:1 molar ratio of the egg PC and PA and cholesterol (or a 16:2:1 weight ratio). Make a chloroform stock solution of the 8:1:1 mixture, at a concentration of 50 mg PC per 6 mL; this limits the lipids' exposure to air, and simplifies the vesicle making procedure. Store under nitrogen in the refrigerator (0 - 5 °C); do not leave the solution at room temperature more than 5 minutes at a time.

Transfer 6 mL (50 mg PC) of the lipid/cholesterol solution to a 50 mL round-bottom flask, and immediately evaporate to dryness on a rotary evaporator. Remove all traces of chloroform from the lipid film by drying under vacuum overnight at ambient temperature, or for four hours with the flask in a 50 °C water bath. After the film is completely dry, add 6 mL of anhydrous diethyl ether to the flask, and quickly dissolve the dried lipid film. Add 2 mL of internal buffer. Stopper the flask with a nitrogen filled balloon to reduce the lipids' exposure to air.

Sonication of the lipid/ether/buffer solution mixes this "water in oil" mixture to homogeneity. Once the sonicator is tuned (13 mm tip), apply 2

second pulses (at 50 % duty cycle and 5 power output) until the mixture is translucent gray. Stopper the flask with the nitrogen balloon. Reverse evaporation of the solution yields mostly large unilamellar vesicles with a high percentage of buffer entrapment. Remove the ether from the mixture by slow rotary evaporation, warming the flask with a 25 °C water bath. As the ether begins to evaporate, the solution coats the sides of the flask; further evaporation promotes spontaneous bubbling. This bubbling continues for about 5 minutes, until almost all the ether has evaporated; the system goes through a gelatinous phase, then becomes a liquid. Add 3 mL of the external buffer, and continue rotary evaporation for half an hour at a slightly reduced vacuum.

The filtration unit sizes the vesicles by forcing the vesicle solution first through a 1 μm Nucleopore filter, then a 0.4 μm filter. Using a 16 gauge 10 mL disposable syringe collect the vesicle solution, draw a further 2 mL of air, and inject the vesicle solution into the first cell. Use 10 psig nitrogen gas to force the vesicle solution through the first filter. Higher pressure may be required to force the solution through the second filter. The slower the filtration, the better the sizing of the vesicles; have the solution filter at 1 or 2 drops per second.

Equilibrate the Sephadex G-25M disposable columns with 10 mL of the external solution. Load all of the vesicle solution. There will be three fractions: 1) multi-lamellar vesicles (MLVs); 2) large unilamellar vesicles (LUVs) (the desired fraction); and 3) small lipid aggregates and excess internal

solution. There is a dead volume of 2.5 mL, and the first 15 drops of the vesicle band must also be discarded (contains mostly MLVs.) Watch for a cloudy "doughnut" to form in the clear eluent in the receiving vial - this is the front of the vesicle band. Collect the next 4.5 mL of vesicles.

The prepared vesicles can be made and used on the same day, or used for the next two days. Batch-to-batch reproducibility is a problem, and within one batch there are differences noted for use over the 72 hour usable lifetime of the vesicles. It appears best to use the entire batch within 36 hours of its preparation.

The pH-stat Experiment

Valinomycin, carbonylcyanidetrifluorom ethoxyphenyl-hydrazone (FCCP), gramicidin D, Triton X-100, and the sulphate salts of potassium, sodium, lithium, rubidium, and cesium, were purchased from Sigma/Aldrich.

Valinomycin, FCCP, and gramicidin D were dissolved in methanol, channel mimics were dissolved in methanol (compounds with thioglucose head-groups) and tetrahydrofuran (compounds with thiopropanol and thioacetate head groups). Triton X-100 was diluted by a factor of 20 with D^3 water.

The following is a brief summary of the protocol used in this study. The vesicles were prepared with an entrapped buffer at pH 6.6. The buffer was composed of Bis-Tris, which was observed not to permeate nor be transported through the membrane. A one pH unit gradient was imposed by addition of

the choline hydroxide titrant solution. A cation concentration gradient was created by adding a cation sulphate salt solution to the system. The transporter was then added, and the pH and cation gradients began to collapse. If necessary, a proton gradient decoupler (FCCP) is added if the transporter cannot achieve antiport translocation of the protons and cations. As the gradients collapse, the protons are released into the bulk solution and the pH drops. The titration instrumentation continuously adds sufficient choline hydroxide titrant to increase the pH to its initial point. The volume of base added versus time data was accumulated until the transport event was complete. The remaining protons were then released into the solution through lysis of the vesicles with Triton X-100, and were subsequently titrated. The data provided a rate constant for the release of protons from the vesicles which correlated with the translocation of the cations across the membrane.

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